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THE INFLUENCE OF RATE AND TEMPERATURE CHANGE AND OF VARIOUS CARDIAC DRUGS ON RHYTHMICITY, CONTRACTILITY, AND THE REFRACTORY PERIOD OF THE TURTLE HEART

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Received for publication January 11, 1934

The observations here reported were made during an investigation which had for its object the correlation of cardiac rhythmicity, and of the refractory period, height of contraction, and duration of systole. It was thought that a comparison of these phases of cardiac activity under different physiological conditions and under the influence of various drugs known to act on heart muscle might perhaps throw further light on the interrelation of these different factors. It seemed especially desirable to ascertain whether or not the inverse ratio of rate of beating and refractory period seen under physiological conditions would still hold when rate change was produced by drug action. The present report is confined to experiments made on the heart of the turtle Pseudomys elegans as this series is more complete, but a sufficient number of observations have been made on mammalian cardiac tissue to indicate that these general conclusions derived from the cold blooded heart are applicable to the higher tissue, that in neither is there necessarily any constant relation between the rate of beating, refractory period, and height of contraction.

The method may be described briefly. The combined auricles or strips from the ventricle were used and the preparation was suspended in a bath of Ringer's solution which was buffered with a phosphate mixture and had a pH of 7.2, and through which oxygen bubbled freely. Contractions were recorded by a light lever with a spring support. Rhythmicity was, of course, studied in the spontaneously beating preparation. For the other observations the heart was rhythmically driven, and the influence of temperature and of various drugs was observed under constant rates of beating. To measure the height and duration of systole the movements

of a fine pointer attached to the vertical arm of the lever were photographed by means of an electrocardiograph camera. Vertical time lines were recorded at 1/12 second intervals by a spoke attached to the shaft of a synchronous motor. Measurements were made with the aid of a lens and are thought to be sufficiently accurate for comparative purposes. Height of contraction could be readily measured within 0.3 mm., while the duration of systole was estimated within approximately 1/48 second. Rhythmic stimulation was obtained by break shocks from a Lewis rotary contact maker and the refractory period was determined by using this same apparatus with a second pair of contact makers, according to a plan which has already been described (7). It is understood that this method measures the effective and not the absolute refractory period, a distinction which has been emphasized by Lewis and Drury (5). This measurement of the effective refractory period undoubtedly includes conduction factors, but it is probably of greater importance when one is studying the contraction as a whole. However, differences in the effective and absolute refractory periods will not affect the general conclusions derived from these experiments, as will appear later.

Effect of rate. In 1913, Mines (6) showed that the refractory period of the ventricle of the frog heart shortened as the rate of beating was raised. Likewise, in these experiments the refractory period of the turtle auricle and ventricle shortened when the rate of beating was increased. According to Dale and Drury (3), this inverse ratio of rate and refractory period appears to be a property of cardiac muscle. As the rate of beating was increased there followed progressive diminution in the height of contraction. However, this decreased contraction at high rates of beating results in part from stimulation of vagal endings. For example, in one experiment, doubling the rate (from 36 to 72 beats per minute) caused a decrease of 49 per cent in height; following the addition of atropine to the bath, the decrease in height of contraction was only 29 per cent for the same rate change. In this same experiment, the refractory period was reduced 28 per cent before atropine and 25 per cent after the auricle had been atropinized. It was quite apparent that the vagus exercised much less influence on the refractory period than on the height of contraction. The duration of systole also varied inversely with the rate of beating; in general, doubling the rate reduced systole by approximately 30 per cent. Observations made before and after atropine indicated that the vagus has no influence on the duration of auricular systole. Ventricular strips responded to rate changes as did the auricles, except that there did not appear to be any vagal influence in the ventricle, and in each experiment the measurements before and after atropinization agreed within the limits of the methods used.

Temperature. Inasmuch as the height of contraction was usually maxi-

mum at room temperature, 21° to 25°C., the values obtained at room temperature were taken as standards. Observations were made on the auricles at temperatures varying from 14° to 33°C. Lowering the temperature slowed the rate, and when the muscle was driven at a constant rate the height of contraction decreased and both the refractory period and the duration of systole were lengthened. When the temperature was raised rhythmicity increased and the refractory period and duration of systole were shortened. In several experiments extrasystoles appeared with a bath temperature of 32° or higher. An increase of temperature of 5° above room temperature (usually 21°C.) appeared to have less effect on the height of contraction than a corresponding decrease in temperature. For an increase of 4° to 6° contraction height changed only slightly, and in various experiments it was seen to increase, decrease, or remain unaltered. At temperatures of 30° to 33°C. a definite decrease in height of contraction was usually observed, while normal rhythmicity increased and the refractory period and the duration of systole became shorter. The changes that resulted from varying the temperature were uninfluenced by atropinization.

Oxygen want. Stopping the supply of oxygen to the bath containing the suspended auricles for periods of 9 minutes in two experiments and for 20 minutes in a third was followed in each instance by a decrease of 30 per cent in the height of contraction, but with no appreciable change in either rhythmicity or the refractory period. The duration of systole was somewhat shortened, possibly because of the reduced contraction.

Atropine. The most constant action of atropine was to prolong slightly the refractory period of auricular muscle; this appeared to be more pronounced at the slower rates of beating. In a single ventricular strip slight lengthening also occurred. The effect on contraction was inconstant and such increase or decrease as did occur was never great. In the majority of experiments the duration of systole remained unchanged. A slight decrease in normal rhythmicity was usually observed, but this cannot for certain be attributed to the drug for the rate always tended to decline as the experiment progressed.

Epinephrine. This substance uniformly accelerated the normal rate of beating, increased the height of contraction, and shortened the refractory period. There occurred a slight increase in the duration of systole, which may have been related to the greater contraction. The action on the auricles and on ventricular strips was similar.

Acetylcholine. This drug slowed the natural rate of beating, decreased the height of contraction, and shortened both the refractory period and the duration of systole. The action was similar for both auricle and ventricle and was entirely prevented by the addition of atropine to the bath.

Adenosine. The effect of adenosine on the turtle heart has been re-

cently reported (7). When there is a definite effect the results are similar to those that have just been described for acetyl choline. But adenosine differs from acetyl choline in that the response is not affected by the presence of atropine in the bath.

Quinidine. The action of quinidine was purely depressant. The normal rate of beating was slowed, contraction height decreased, the refractory period was prolonged, and the duration of systole was lengthened, even when there was considerable decrease in the height of contraction.

Caffein. Many observations were made on the action of caffein, but the effects were inconstant and at times different results were obtained by repeating the experiment with the same preparation. In view of the work of Cheney (2) which showed that the action of caffein in delaying fatigue in striated muscle was dependent on the buffer, both sodium bicarbonate and phosphate mixtures were used to buffer the Ringer solution, but in these experiments the choice of buffer did not appear to exert any influence on the caffein effect. Caffein alkaloid in concentrations of 1:10,000 and 1:5,000 sometimes produced considerable increase in the height of contraction when it had almost no effect on rhythmicity or on the refractory period. For present purposes, it is sufficient to point out the lack of any constant relation between the influence on the normal rate of beating and the refractory period; the latter has been observed to shorten or remain unchanged when rhythmicity was appreciably depressed, as well as when it was enhanced by caffein.

Digitalis. To study the action of digitalis water soluble digifoline was used, in concentrations varying from 1:10,000 to 1:5,000. The auricle alone was tested. Extrasystoles were frequently produced, but when the rhythm continued regular the effect on its rate was comparatively slight, and both increases and decreases were recorded. The effect on the height of contraction was likewise variable. After one to two hours alternation frequently appeared. The refractory period was lengthened, except in one experiment in which shortening occurred, with an increase of 47 per cent in the normal rate of beating.

Veratrine sulphate. Veratrine increased the natural rate of beating of both auricles and ventricular strips. With small concentrations of the drug, 1:100,000 to 1:75,000, the height of contraction often increased, both when the muscle was driven and when it beat normally, even at a more rapid rate. Higher concentrations, 1:50,000 and greater, caused alternation, or irregularity in the height of contraction and finally fibrillation. Delayed relaxation, such as occurs in skeletal muscle was observed with the higher concentrations. The duration of systole remained quite constant, even when the amplitude of contraction was considerably increased. The refractory period continued to lengthen during the course of the experiment; increases from 30 to 50 per cent were recorded.

CONCLUSION

From these studies it appears that in the turtle heart rhythmicity, contractility, and the refractory period are capable of varying independently of one another. The inverse ratio of rate of beating and refractory period which has been considered a property of cardiac muscle, was maintained when rate increases were produced by rhythmic stimulation, and likewise, when rhythmicity was altered by varying the temperature. However, this ratio did not hold under the influence of certain drugs, especially adenosine, acetyl choline and veratrine. If one accepts the criticism of Drury and Love (4) that although veratrine may prolong the effective refractory period it shortens the absolute refractory period then the usual inverse ratio for rate and refractory period may be considered to hold under this drug. But it will then be necessary to accept the same criticism made by Lewis and Drury (5) for quinidine and admit shortening of refractory period when rhythmicity is definitely depressed, and thus the action of this drug which is purely depressant when the effective refractory period is considered becomes irregular and the normal rate: refractory period ratio is disturbed.

The height of contraction altered with the rate of beating, but the decline at high rates in the case of auricular muscle was due in considerable part to the rapid stimulation of vagal endings. Under drugs, those which constantly depressed rhythmicity, acetyl choline, adenosine, and quinidine, diminished the height of contraction height. However, caffein at times increased contraction without producing any change in rate or refractory period. Increasing the temperature to 30° to 33° caused a diminution in contraction while rhythmicity increased and the refractory period shortened. Oxygen want at a constant rate of beating caused decreased contraction height without change in refractory period or normal rhythmicity. This may be explained in the light of the work of Blum (1) by assuming that interference with the velocity of recovery has resulted from oxygen deprivation even though the interval betweeen stimuli was kept constant.

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HEMOGLOBIN PRODUCTION FACTORS IN THE ANEMIC HORSE LIVER

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When it was established (8) that beef liver contained abundant materials which the anemic dog could convert into hemoglobin and red cells under the stress of experimental anemia one could hardly avoid speculation as to the probable depletion of this rich store of hemoglobin production factors in the liver due to long continued anemia. We have reported recently (7) that the human liver in anemia due to blood loss contains hemoglobin production factors in amounts rated as low normal. We were greatly surprised by this observation and at first were inclined to explain these findings as due to some unknown variables. It did seem obvious that the human organism could replete this liver store of hemoglobin production factors in an extraordinarily successful manner.

The ideal experiment would concern some large animal made anemic by blood loss. The anemia would then be continued for some weeks by means of bleeding before the animal was killed for biological assay of the liver. At first we tested some antitoxin horses which were anemic at time of death, this liver material being furnished us through the interest of Mr. G. B. Walden and Eli Lilly & Company. As this liver material seemed to be somewhat below normal potency (table 3) we were still dissatisfied as toxin injections were confusing factors and we were unable to control accurately the anemic condition of these horses.

Finally in our own laboratory a horse was made anemic by bleeding and kept anemic for 8 weeks at a level of 50 per cent hemoglobin before being killed. This liver material was carefully tested with adequate controls as given in tables 1 and 2 below. It is obvious that the *anemic horse liver* contains no less of the hemoglobin production factors than do the normal controls.

Evidently the normal liver guards its reserve of hemoglobin production factors with the utmost care and repletion must be prompt and effective. In human cases of pernicious anemia and aplastic anemia we have reported (7) a *surplus accumulation* of these hemoglobin producing factors in the liver due possibly to the lack of outlet for this material as the body

cannot fabricate red cells due to a deficiency factor (pernicious anemia) or to lack of red marrow (aplastic anemia). Only in severe liver disease with signs of failure of liver function (3) do we find a significant fall in the concentration of hemoglobin production factors in the human liver.

METHODS. The care of animals and general technique employed in the anemia colony have been described in detail elsewhere (4, 5). The iron analysis was done on samples as described by Kennedy (2) and the figures given as milligrams per cent of Fe appear in the tables. Fresh weight of the organ was used. It is obvious that these figures do not represent quantitatively the amount of *tissue iron* as the amount of contained blood is a variable which is not controlled in this material. Salmon bread as fed contains 4.7 mgm. per cent iron which figure is an average from many analyses. Canned salmon as fed contains only small amounts of iron—0.8 mgm. per cent. These values are included in the total daily Fe intake as given in the tables. Occasional small amounts of meat extract may be added to a diet which is not eaten completely and this is not noted in the tables. Adequate control observations show that these *small* amounts of meat extract have no demonstrable effect upon hemoglobin and red cell production in anemia.

In figuring the true output of hemoglobin given in the tables as "total net output" we allow 2 grams per week as the usual base line output on salmon bread during control periods. If there is a difference in the hemoglobin levels before the liver period begins and at the end of the after period which includes the carry over from the liver diet, correction is made on the basis of 1 gram hemoglobin for each per cent in the blood hemoglobin level.

EXPERIMENTAL OBSERVATIONS. *Rochester horse—anemia experiment.* This animal was an adult farm horse probably beyond middle age but healthy and strong. Its weight was estimated at 1050 pounds and the blood volume presumably about 9 per cent of body weight or 45 litres. The horse was given the usual daily feeding of alfalfa hay and continued in good clinical condition until death. The initial hemoglobin level was 101 per cent on February 11, 1931 (100 per cent hemoglobin equals 13.8 gm. per 100 cc. blood). Between February 11 and April 20 by means of interval bleedings about 3 times per week, the blood hemoglobin level was reduced to 45 per cent hemoglobin. A total of 55 litres of blood was removed during this period. Blood was removed readily by means of a large needle placed in the jugular vein.

The anemia period lasted from April 20 to June 18, 1931, and the blood hemoglobin level during this 2-month period was maintained by suitable bleeding between 42 and 56 per cent hemoglobin. During this period of continuous anemia the horse was bled 42 litres to maintain the desired hemoglobin level. When we compare blood volume and hemoglobin

volume as estimated for this horse it is obvious that this horse holds less hemoglobin producing factors in reserve than does the normal dog. It has been shown (1) that a 2-month period of anemia due to blood loss will remove all the surplus iron reserve storage from the liver in the dog. The same is probably true for the horse as the iron analyses indicate a very low level for this anemic liver.

The horse was fasted for 2 days before death so that hemoglobin construction materials coming into the liver from the food might be excluded.

The horse was killed on June 18, 1931, and an autopsy done promptly. The viscera were all normal in gross but for anemia. The blood hemoglobin at this time was 47 per cent. The liver weighed 4870 grams, spleen 750 grams and both kidneys 1105 grams. The biological analysis of the hemoglobin producing factors is given in tables below.

Histological sections: Liver shows normal cells in all its lobules but a few fat droplets are visible. Many fine yellow pigment granules are seen within the liver cells. From the iron analyses it is obvious that this pigment is non-iron containing and therefore probably related to the lipochrome pigment found in human livers in the last two decades of life. The portal tissue contains small clusters of mononuclear cells. The *spleen* shows a normal structure and no pigment. Clusters of eosinophiles are seen in the spleen pulp. The *kidney* is normal in all respects.

The antitoxin horse liver obtained from Eli Lilly & Company represented a typical case as observed in their laboratories. The horse had been receiving regular toxin injections with interval bleedings over a period of many months. The decline in general health was very gradual as is usual in these horses. One month before death the horse showed a hemoglobin level of approximately 40 per cent. Autopsy showed some inflammation of endocardium and pericardium. The liver was enlarged and friable—for analysis and biological assay of this liver see table 3.

Table 1 shows that the anemic horse liver is no less potent than the normal horse or pig liver in promoting new hemoglobin and red cell production in the anemic dog. In fact the anemic liver seems to contain if anything somewhat more of the *organic* food factors than do the controls particularly if we make some allowance for the iron content of these three types of liver. The anemic liver contained 8.6 mgm. per cent Fe, the normal horse control liver 68.4 mgm. per cent Fe, and the normal pig liver the usual level of 19 mgm. per cent Fe. When we figure in all the food iron we see that the normal control horse liver diet contains five times as much as the diet including the anemic horse liver. The pig liver control diet contains more than twice as much iron per day intake as the anemic horse liver diet. This pig liver diet gives a total hemoglobin production of 86 grams which corrected for the excess intake gives the true comparative total output as 53 grams hemoglobin. This is distinctly below the normal or anemic horse liver diet output (table 1).

Table 2 shows the second test of the anemic Rochester horse liver. There are minor differences but the evidence is again strong that the anemic liver contains no less of the hemoglobin producing factors than do the non-anemic controls. The control horse liver is unusually low in its Fe content

TABLE 1

Anemic horse liver and controls

DIET PERIODS 1 WEEK EACH	FOOD CONS.	DAILY Fe INTAKE	WT.	PLASMA VOL.	RBC	COLOR INDEX	BLOOD HB. LEVEL	HB. REMOVED BLED	TOTAL NET HB. OUTPUT
Food, grams per day	*per cent*	*mgm.*	*kgm.*	*cc.*	*mil.*		*per cent*	*grams*	*grams*
Dog 26–164			Anemic horse liver						
Br. 350, salm. 150, Kl. 40...	94	17	13.4	825	4.5	0.50	45	1.3	
Liver 165, br. 300, Kl. 40...	97	28	13.5	730	6.4	0.58	63	45.4	
Liver 200, br. 300, Kl. 40...	100	31	12.9	714	6.0	0.53	49	36.6	
Br. 300, salm. 150, Kl. 40...	100	15	13.1	782	4.7	0.52	45	12.3	
Br. 300, salm. 150, Kl. 40...	100	15	13.5	803	4.6	0.53	49	1.4	92
			Normal horse liver						
Br. 300, salm. 150, Kl. 40...	100	15	13.5	803	4.6	0.53	49	1.4	
Liver 165, br. 275, Kl. 40...	100	126	13.2	721	6.3	0.59	63	43.7	
Liver 200, br. 300, Kl. 40...	100	151	13.0	713	5.5	0.59	44	24.8	
Br. 325, salm. 150, Kl. 40...	100	16	12.9	791	4.2	0.52	44	1.3	
Br. 325, salm. 150, Kl. 40...	95	16	12.7	726	4.8	0.55	48	12.1	
Br. 325, salm. 150, Kl. 40...	100	16	13.2	777	5.1	0.53	48	12.3	83
			Normal pig liver						
Br. 375, salm. 75, Kl. 25....	100	19	12.6	780	4.3	0.48	41	1.1	
Liver 300, br. 325.....	100	72	12.7	713	5.9	0.57	55	29.9	
Liver 300, br. 325.....	100	72	12.8	731	6.1	0.69	51	51.0	
Br. 375, salm. 75, Kl. 25....	100	19	12.8	813	4.4	0.58	37	13.3	(86)
Br. 375, salm. 75, Kl. 25....	97	19	13.0	825	3.6	0.56	40	1.1	53

Br. = salmon bread; Salm. = canned salmon; Kl. = "Klim," a skim milk powder.

—15.6 mgm. per cent in contrast to the control horse liver in table 1—68.4 mgm. per cent Fe. This is probably due to dietary factors or possibly to diseased conditions about which we have no knowledge. Clinically the horse was considered normal. The liver was normal in gross. The

control pig liver again is below the horse liver when we compare the total net hemoglobin output corrected for liver diet intake—total hemoglobin

TABLE 2

Anemic horse liver and controls

DIET PERIODS 1 WEEK EACH	FOOD CONS.	DAILY Fe INTAKE	WT.	PLASMA VOL.	RBC	COLOR INDEX	BLOOD HB. LEVEL	HB. REMOVED BLED	TOTAL NET HB. OUTPUT
Food, grams per day	*per cent*	*mgm.*	*kgm.*	*cc.*	*mil.*		*per cent*	*grams*	*grams*
Dog 27-239			Anemic horse liver						
Br. 450, salm. 75, Kl. 30....	100	22	14.2	833	4.0	0.56	45	1.4	
Liver 175, br. 250, Kl. 30...	100	27	13.7	738	6.6	0.52	52	27.2	
Liver 175, br. 250, Kl. 30...	100	27	13.2	723	5.5	0.52	55	14.3	
Br. 400, salm. 75, Kl. 30....	100	20	13.5	727	6.0	0.52	45	28.7	
Br. 450, salm. 75, Kl. 30....	82	22	13.3	772	4.7	0.49	46	1.4	
Br. 300, salm. 150, Kl. 40...	74	15	13.1	768	5.8	0.55	41	26.4	
Br. 300, salm. 150, Kl. 40...	100	15	13.6	789	4.7	0.45	42	1.2	84
			Normal horse liver						
Br. 350, salm. 100, Kl. 40...	100	17	13.9	827	4.8	0.48	46	1.2	
Liver 175, br. 200, Kl. 30...	100	36	13.6	736	6.0	0.49	53	35.5	
Liver 175, br. 250, Kl. 30...	100	39	13.6	742	5.7	0.47	59	21.4	
Br. 350, salm. 100, Kl. 40...	100	17	13.7	769	5.7	0.48	44	22.4	
Br. 350, salm. 100, Kl. 40...	100	17	13.8	809	4.9	0.50	42	12.9	
Br. 350, salm. 100, Kl. 40...	100	17	13.8	809	4.9	0.46	45	1.4	83
			Normal pig liver						
Br. 350, salm. 100, Kl. 40...	100	17	14.3	838	4.8	0.50	48	1.4	
Liver 225, br. 200, Kl. 40...	75	52	13.5	720	6.3	0.57	53	39.2	
Liver 225, br. 250, Kl. 40...	92	55	13.4	734	5.4	0.51	51	24.1	
Liver 140, br. 300, Kl. 40...	100	41	13.3	768	5.4	0.52	55	37.0	
Br. 350, salm. 150, Kl. 40...	100	17	13.6	742	4.7	0.59	41	25.6	(116)
Br. 375, salm. 150, Kl. 40...	100	19	13.7	815	5.0	0.47	47	1.3	66

Br. = salmon bread; Salm. = canned salmon; Kl. = "Klim," a skim milk powder.

production 116 grams corrected to compare with the amount horse liver fed is 66 grams hemoglobin.

Table 3 shows 2 experiments with the Lilly antitoxin horse liver. In

these experiments the anemic dog received the standard feeding of liver (300 grams daily) for 2 weeks and the total hemoglobin output due to the liver in the two dogs was 81 and 87 grams. This is distinctly below normal control values for these two dogs. Pig liver control figures for 24–45 are about 110 grams hemoglobin and for 24–59 about 140 grams hemoglobin. When we compare these antitoxin livers (table 3) with tables 1 and 2 (anemic livers due to blood loss) we see evidence that the antitoxin anemic

TABLE 3

Anemic horse liver—antitoxin horse

DIET PERIODS 1 WEEK EACH	FOOD CONS.	WT.	PLASMA VOL.	RBC	COLOR INDEX	BLOOD HB. LEVEL	HB. REMOVED BLED	TOTAL NET HB. OUTPUT
Food, grams per day	*per cent*	*kgm.*	*cc.*	*mil.*		*per cent*	*grams*	*grams*
Dog 24-45								
Br. 400, salm. 75	100	20.5	1240	3.9	0.55	43	1.3	
Liver 300, br. 300	100	20.5	1224	4.7	0.48	45	1.3	
Liver 300, br. 300	100	20.6	1142	5.5	0.57	71	38.7	
Br. 400, salm. 75	100	20.4	1183	5.6	0.59	60	32.4	
Br. 400, salm. 75	100	19.9	1045	5.7	0.51	45	25.6	
Br. 400, salm. 75	100	19.7	1214	3.3	0.53	35	1.0	81
Dog 24-59								
Br. 450, salm. 75	100	15.9	1060	3.9	0.58	45	1.4	
Liver 300, br. 300	100	15.6	932	6.4	0.54	51	41.4	
Liver 300, br. 300	100	16.1	958	5.2	0.49	51	19.6	
Br. 450, salm. 75	100	15.7	982	5.2	0.56	50	31.8	
Br. 450, salm. 75	100	15.6	1058	3.7	0.62	46	1.4	
Br. 450, salm. 75	100	15.8	1050	4.6	0.50	46	1.3	87

Br. = salmon bread; Salm. = canned salmon.

liver contains decidedly less material out of which hemoglobin can be formed. We do not know the iron content of these antitoxin livers. These observations are not as significant as those recorded in tables 1 and 2 because of unknown variables. Two more experiments of identical nature were done with the antitoxin livers and gave results identical with those recorded in table 3.

Table 4 shows that the anemic horse spleen contains slightly less of the hemoglobin production factors than does the control normal horse spleen.

This may be due to the contained iron as it is easily demonstrated that long continued bleeding will always reduce markedly the storage of iron in the spleen. The control spleen contained 46 mgm. per cent Fe and the figures for the anemic spleen are very low but not quite certain—we may safely conclude that the anemic spleen contained less iron. Histologically it contained no iron staining pigment.

These figures for horse spleen are unusually high—70 and 82 grams hemoglobin production. Two other experiments with normal horse

TABLE 4

Anemic horse spleen and control

DIET PERIODS 1 WEEK EACH	FOOD CONS.	DAILY Fe INTAKE	WT.	PLASMA VOL.	RBC	COLOR INDEX	BLOOD HB. LEVEL	HB. REMOVED BLED	TOTAL NET HB. OUTPUT
Food, grams per day	*per cent*	*mgm.*	*kgm.*	*cc.*	*mil.*		*per cent*	*grams*	*grams*
Dog 26-102		Anemic horse spleen							
Br. 300, salm. 125, Kl. 40...	100	15	13.4	789			47	1.4	
Spleen 107, br. 300, Kl. 40..	100		13.7	766	5.4	0.53	50	25.4	
Br. 325, salm. 100, Kl. 40...	100	16	13.7	745	5.2	0.55	46	25.8	
Br. 350, salm. 100, Kl. 40...	95	17	13.6	777	5.6	0.55	39	27.2	
Br. 350, salm. 150, Kl. 40...	88	17	13.9	806	4.7	0.48	45	1.3	70
		Normal horse spleen							
Br. 275, salm. 200, Kl. 40...	96	15	14.3	838	3.8	0.54	41	1.2	
Spleen 107, br. 275, Kl. 40..	99	63	14.0	747	6.7	0.56	47	42.7	
Br. 250, salm. 200, Kl. 40...	100	14	14.4	786	5.8	0.49	40	24.4	
Br. 250, salm. 200, Kl. 40...	100	14	14.6	812	5.5	0.48	32	20.3	
Br. 250, salm. 200, Kl. 40...	90	14	14.7	888	4.1	0.51	42	1.2	82

Br. = salmon bread; Salm. = canned salmon; Kl. = "Klim," a skim milk powder.

spleen give strictly comparable results. In earlier experiments the beef spleen (6) was found to be low in potency or about one-half the standard liver potency. It is possible that the spleens of different animals like the livers (second paper) contain different amounts of hemoglobin producing factors.

Table 5 shows that the anemic horse kidney contains the same amount of hemoglobin producing material as does the normal horse kidney. The normal beef kidney (table 5) is just a little less potent. Pig kidney as tested on standard dogs rates about 90 per cent of pig liver potency. This

figure for pig kidney is about the same as for the anemic or normal horse kidney. The iron content of kidney tissue is low—3.6 mgm. per cent for the anemic horse kidney—5.7 mgm. per cent for the normal horse kidney—4.7 mgm. per cent for the normal beef kidney. Obviously the iron content is not a feature of the kidney reaction.

TABLE 5

Anemic horse kidney and controls

DIET PERIODS 1 WEEK EACH	FOOD CONS.	DAILY Fe INTAKE	WT.	PLASMA VOL.	RBC	COLOR INDEX	BLOOD HB. LEVEL	HB. REMOVED BLED	TOTAL NET HB. OUTPUT
Food, grams per day	*per cent*	*mgm.*	*kgm.*	*cc.*	*mil.*		*per cent*	*grams*	*grams*
Dog 29–67	Anemic horse kidney								
Br. 375, salm. 125, Kl. 40...	79	19	15.1	902	5.2	0.47	49	1.3	
Kidney 155, br. 275.....	96	19	15.3	836	6.5	0.47	45	24.4	
Br. 350, salm. 125, Kl. 40...	100	17	15.5	936	5.5	0.43	47	1.3	
Br. 350, salm. 125, Kl. 40...	100	17	15.6	915	5.4	0.49	50	26.3	47
	Normal horse kidney								
Br. 325, salm. 125, Kl. 40...	100	16	16.2	926	4.9	0.48	47	1.4	
Kidney 155, br. 250.....	100	21	15.6	830	6.6	0.51	53	28.5	
Br. 325, salm. 125, Kl. 40...	100	16	15.8	878	5.5	0.52	41	21.5	
Br. 325, salm. 125, Kl. 40...	100	16	16.1	962	4.7	0.46	43	1.2	41
	Normal beef kidney								
Br. 325, salm. 125, Kl. 40...	100	16	16.3	974	4.5	0.41	37	1.1	
Kidney 155, br. 250.....	100	19	16.0	899	5.8	0.47	58	13.6	
Br. 325, salm. 100, Kl. 40...	100	16	16.5	956	5.0	0.49	48	20.3	
Br. 325, salm. 100, Kl. 40...	100	16	16.3	974	5.2	0.43	45	1.2	37

Br. = salmon bread; Salm. = canned salmon; Kl. = "Klim," a skim milk powder.

Discussion. Why does the liver in the anemic animal contain the usual store of material out of which new hemoglobin and red cells can be fabricated by the standardized anemic dog? We cannot answer this question to our own satisfaction but make the following suggestions. Possibly the anemia (horse) is too mild and not continued over a sufficient period. Human livers in long continued, very severe anemia due to blood loss do

show a slight decrease in this store of hemoglobin producing material but even so this change is only slight. When the marrow is incapable of function (aplastic anemia) this material does pile up in the liver and may rise to double the normal value.

Possibly one might argue that the liver is busy with the manufacture of parent hemoglobin building material and during this moderately severe anemia due to blood loss this mechanism is accelerated and no depletion of reserve is permitted in spite of a severe drain due to hemoglobin loss by bleeding. In fact if we consider the observation that the iron content is much decreased in these anemic livers and iron has a definite influence upon hemoglobin regeneration, we may properly claim that the anemic horse liver actually contains more of the *organic factors* which promote hemoglobin regeneration than does the non-anemic control liver. We therefore may be driven to the admission that the liver in moderate anemia (compared with controls) does contain much less iron and therefore *more organic factors* which promote hemoglobin regeneration. The activity of the horse liver stimulated by the lack of hemoglobin (anemia) presumably is responsible for this increased production and storage of the organic factors present in the anemic liver as demonstrated by diet tests in standard anemic dogs.

SUMMARY

Moderately severe anemia due to blood loss in the horse lasting 2 to 3 months does not deplete the hemoglobin producing factors present in the liver.

The store of iron is depleted and making allowance for this iron depletion we have evidence that the *organic factors* promoting hemoglobin regeneration may be somewhat *increased* in this anemic horse liver.

In the anemic antitoxin horse liver there is definite depletion of the hemoglobin producing factors.

In the anemic spleen the iron store is depleted and probably because of this the hemoglobin producing factors are somewhat less than control normal spleen tissue.

The anemic kidney runs parallel to the normal controls as measured by hemoglobin production in the standard anemic dog.

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HEMOGLOBIN PRODUCTION FACTORS IN NORMAL LIVERS OF DOMESTIC ANIMALS

Horse Liver Rates High and Beef Low

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Our first experiments which showed that liver had a significant effect upon the regeneration of hemoglobin and red cells in anemic dogs were done in California (2). Beef liver was used at that time because more readily available. When the anemia colony was moved to Rochester there were many variables which soon were placed under control and we changed from the short anemia period to the long continued severe anemia due to blood loss which has been our standard procedure during the past 11 years. At that time we changed to pig liver as our standard test material for high hemoglobin output.

On various occasions as opportunity offered we tested various types of mammalian liver and it soon became apparent that there were differences due either to individual or species variables. A glance at the tables below shows that horse liver is almost twice as rich as beef liver in the hemoglobin producing factors per unit weight of fresh tissue. If we rate pig liver as 100 per cent we find that beef liver averages 70 per cent and horse liver 131 per cent.

Experimental observations. References to various method procedures are given in the preceding paper. The weights for liver as given in various tables refer to the original fresh weight whether the liver is subsequently cooked or fed as a powder (table 22). In certain tables (table 23) the amount of the tested liver was not the same as the daily intake of the pig control liver and in computing the *potency ratio* the proper correction was introduced. In two or three experiments pig kidney was used as a standard but the figures were corrected to the liver value by the established ratio of liver to kidney potency of 100 to 90. This corrected figure was then used to establish the potency ratio.

During the past 10 years we have enjoyed the cordial coöperation of the Rochester Packing Company. They have supplied at nominal cost the pig liver material used in all our experiments and we feel indebted to them for much valuable material and assistance. We are indebted to

Dr. C. H. Watkins of the Mayo Clinic for the fetal calf liver powder. Some of this was prepared in the Abbott Company laboratories and some in the laboratories of the Mayo Clinic. The fresh fetal calf liver material came to us through the interest of Mr. Walden and Eli Lilly & Company. The reindeer livers were furnished through the courtesy of Dr. Louise Stanley, U. S. Department of Agriculture, and the Lomen Reindeer Corporation, and came to us as fresh refrigerated material in perfect condition. The rabbit livers were furnished through the interest of Dr. Louise Stanley and Mr. F. G. Ashbrook of the U. S. Department of Agriculture, by the Lehigh Packing Company.

Table 21 shows that beef liver on the average contains 70 per cent as much hemoglobin producing factors as does pig liver. The iron analyses

TABLE 21

Normal beef liver

DOG NUMBER	BEEF LIVER			POTENCY RATIO	PIG LIVER		IRON INTAKE DAILY	
	Intake daily	Hb. output 2 weeks	Type		Intake daily	Hb. output 2 weeks	Beef liver	Pig liver
	grams	*grams*		*per cent*	*grams*	*grams*	*mgm.*	*mgm.*
26–102	300	71		76	300	94	21	57
29–65	300	86		73	300	118	21	57
27–234	300	52		43	300	122	21	57
30–116	300	57		58	300	99	21	57
30–115	300	80		91	300	88	21	57
23–1	300	79	Dry	80	300	99	16	57
26–164	300	36	Dry	42	300	86	16	57
26–164	300	64	Dry	74	300	86	11	57
26–164	300	93	Lact.	108	300	86	18	57
26–164	300	51	Bull	59	300	86	13	57
Average.....		67		70		96	18	57

show low values for beef liver and this may be responsible in part for the lower potency ratio of beef liver. The iron analysis for pig liver given as 57 mgm. per 300 grams fresh weight is the average of a large number of determinations done at various times on lot mixtures.

The last four experiments (table 21) show the reaction to *individual beef livers*—two coming from non-lactating cows, one from a lactating cow and one from an adult bull. These were all tested on the same dog to minimize any individual variables. These observations suggest that *lactation* may modify the store of hemoglobin producing factors in the normal liver but there are many factors to be considered and slaughter house material is unsatisfactory as we know little or nothing about the past history of the animal. It is quite possible that this store of hemo-

globin producing materials in the liver may be disturbed in lactation and vary with the extent and duration of milk secretion. We have some evidence from human material (3) that early in lactation this store of hemoglobin producing material may be very low. Later in lactation this store may be repleted and rise above normal (table 21). As we have evidence (1) that there may be a good deal of "ebb and flow" between various serum and organ or tissue proteins, these observations relating to hemoglobin production factors in the liver as related to lactation take on added interest and call for more study of beef material in which the lactation history and diet régime are known.

Table 22 shows that fresh fetal calf liver contains more hemoglobin producing factors than does beef liver. The potency ratio for fresh fetal liver is 124 per cent, adult beef liver 70 per cent and control pig liver 100 per cent. The powdered fetal liver potency ratio is only 58 per cent showing presumably that there is a considerable loss in potency as the organ tissue is dried and powdered. It is fair to say however that the powdered material came from a different source. Unfortunately no iron analyses are available but the material was probably richer in iron than the adult liver material.

TABLE 22

Fetal calf liver

DOG NUMBER	FRESH LIVER		LIVER POWDER		PIG LIVER	
	Intake daily	Hb. output 2 weeks	Intake daily	Hb. output 2 weeks	Intake daily	Hb. output 2 weeks
	grams	*grams*	*grams equiv.*	*grams*	*grams*	*grams*
24–26*	210	42	225	39	225	73
27–234	105	90			300	148
23–1	200	75			300	76
25–23	260	85			300	83
27–233			300	72	300	140
27–240			300	65	300	92
27–241			300	56	300	87
24–45			300	93	450	108

* Dog with chronic nephritis—low hemoglobin output.

It is interesting to note that dog 24-26 (table 22) always shows a low hemoglobin output on standard food materials. This dog has a chronic nephritis which followed a mild attack of distemper and it is probable that these low hemoglobin output figures are to be explained at least in part on the basis of the chronic nephritis—refer to the same dog in tables 25 and 26. We have observed the same low output in dogs with suppurative pyorrhea and chronic endometritis and hope to report more experiments touching this point in the near future.

Table 23 shows that horse liver contains maximal stores of hemoglobin production material when compared with the other domestic animals. The store of iron in the horse liver is likewise high, 86 mgm. per cent of horse as compared with pig liver 57 mgm. per cent. This iron surplus may be in part responsible for the excess hemoglobin production but we

TABLE 23

Normal horse liver

DOG NUMBER	HORSE LIVER		POTENCY RATIO	PIG LIVER		IRON INTAKE DAILY	
	Intake daily	Hb. output 2 weeks		Intake daily	Hb. output 2 weeks	Horse liver	Pig liver
	grams	*grams*	*per cent*	*grams*	*grams*	*mgm.*	*mgm.*
27–239	175	83	122	300	116	27	57
26–164	182	83	160	300	86	125	57
27–234	380	135	87	300	122	59	57
27–235*	290	188	130	300	150	162	57
30–121	170	101	163	300	109	95	57
26–102	310	124	123	300	98	48	57
Average			131			86	57

* Dog hyperactive in hemoglobin production.

TABLE 24

Normal reindeer liver

DOG NUMBER	REINDEER LIVER		POTENCY RATIO	PIG LIVER	
	Intake daily	Hb. output 2 weeks		Intake daily	Hb. output 2 weeks
	grams	*grams*	*per cent*	*grams*	*grams*
25–23	235	70	108	300	83
27–236	263	76	79	260	95
24–59*	300	148	101	300	147
27–233	300	104	85	300	122
27–239	300	90	78	300	116
27–240	300	83	90	300	92
Average			90		

* Dog hyperactive in hemoglobin production.

must keep in mind that the liver iron store may be depleted by bleeding without lowering the store of hemoglobin producing factors (tables 1 and 2, paper I).

Dog 27-235 (table 23) illustrates an individual variable of some interest. This dog has been continuously anemic for $3\frac{1}{2}$ years at the standard level and has shown uniform high hemoglobin output values on all diet factors.

This type of dog we list as "hyperactive" in hemoglobin production and we have observed such dogs throughout a normal life cycle with continuous high hemoglobin production. Some of these dogs have been raised on diets rich in liver after weaning before being made anemic and we suspect this as a causative factor. This individual characteristic usually lasts throughout life. We hope to report in detail at a later date.

TABLE 25

Normal dog liver

DOG NUMBER	DOG LIVER		PIG LIVER		IRON INTAKE DAILY	
	Intake daily	Hb. output 2 weeks	Intake daily	Hb. output 2 weeks	Dog liver	Pig liver
	grams	*grams*	*grams*	*grams*	*mgm.*	*mgm.*
24–26*	225	54	225	73	28	43
29–68	300	132	300	120	37	57
30–114	300	138	300	143	58	57
29–66	300	123	300	103	61	57
Average.....		112		110	46	54

* Dog with chronic nephritis—low hemoglobin output.

TABLE 26

Normal rabbit liver

DOG NUMBER	RABBIT LIVER		PIG LIVER		IRON INTAKE DAILY	
	Intake daily	Hb. output 2 weeks	Intake daily	Hb. output 2 weeks	Rabbit liver	Pig liver
	grams	*grams*	*grams*	*grams*	*mgm.*	*mgm.*
24–26*	225	43	225	73	17	43
26–18	300	124	300	148	23	57
29–67	300	124	300	116	23	57
Average.....		97		112	21	52

* Dog with chronic nephritis—low hemoglobin output.

Table 24 shows that reindeer liver is a little richer in hemoglobin production factors than is beef liver—a potency ratio of 90 per cent compared with 70 per cent for beef liver. Dog 24-59 (table 24) is another dog listed as "hyperactive" in hemoglobin production. This has been a uniform individual factor observed uniformly over a period of continuous anemia lasting $7\frac{1}{2}$ years.

Table 25 shows that normal dog livers have a potency ratio equivalent to the standard pig liver. These livers came from healthy mongrels killed with gas or ether anesthesia. The livers were normal in gross, were

removed promptly and kept in cold storage until used. All samples tested were mixtures and the liver tissue was ground and thoroughly mixed before iron samples were taken and food portions utilized. Under the experimental conditions both the pig and dog are omnivorous and subsist on a mixture of table scraps and milk. Too much emphasis can not be placed on the diet however when we recall that the horse and cow show such divergence in liver potency ratio while their diet may consist almost wholly of hay. However one cannot avoid the suspicion that it should be possible to vary the liver store of hemoglobin production factors by diet control.

Table 26 shows that normal rabbit liver rates a little above beef liver but below the standard pig liver. The rabbit liver potency ratio is about 80 per cent when compared with pig liver as 100 per cent. The rabbit liver is low in iron and compares in this respect with beef liver.

SUMMARY

If we accept as 100 per cent the content of hemoglobin production factors in the normal pig liver, then we are able to show that the *potency ratio* for beef liver will be low (70 per cent) and for horse liver will be high (130 per cent).

Lactation (table 21) may modify the content of hemoglobin production factors in the liver.

Fetal calf liver (fresh) in contrast to beef liver may show a high potency ratio (124 per cent).

Dog and pig livers show equivalent potency ratios.

Reindeer liver contains somewhat more hemoglobin production factors than does beef liver—a potency ratio of 90 per cent compared to 70 per cent for beef.

Rabbit liver contains a slightly higher concentration (80 per cent) of hemoglobin production factors than does beef liver.

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PROTEIN AND ENERGY METABOLISM OF WILD AND ALBINO RATS DURING PROLONGED FASTING

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In a previous study on the metabolism of the wild rat in the Nutrition Laboratory the respiratory quotient, only occasionally determined, was in several instances extremely high just prior to death (Benedict and Petřík, 1930). With other fasting animals a pre-mortal rise in nitrogen excretion or in the carbon-dioxide elimination has been noted, but so far as we are aware, simultaneous observations of the respiratory quotient and the nitrogen excretion during the terminal stages of fasting have not as yet been reported with any species of animal. Hence we subjected a number of wild and albino rats to prolonged fasting, and during these fasts respiratory and urinary studies were made. Observations were also made with a group of rats huddling and separated, primarily to throw light upon the problem of heat loss as affected by exposed surface area and secondarily to note whether huddling minimizes the activity of the rat.

Albino rats were kindly furnished us by Prof. W. E. Castle of the Bussey Institution of Harvard University and by Dr. M. O. Lee of the Harvard Medical School. The wild rats were trapped in a nearby abattoir. The metabolism was measured with an open-circuit respiration apparatus, and samples of the outcoming air were analyzed on the Carpenter gas-analysis apparatus. The degree of repose during each experimental period was graphically recorded.

Basal metabolism of the rat. Our albino rats, 5 females and 3 males (weight range, 92 to 142 gm.), had a basal heat production 17 to 50 hours after food averaging 24.9 calories per 200 grams per 24 hours. Of our nine wild rats only two had their metabolism measured during the early stage of fasting, for we were primarily interested in the physiology of the rat after prolonged fasting. In these two instances (one male weighing 222 gm. and one female of 321 gm.) the heat production 24 hours after food averaged 23.0 calories per 200 grams. Referred to body surface ($S = 9.1 \times w^{2/3}$) the basal heat production of our 3 male albino rats averaged 671 calories and of our 5 female rats, 683 calories. Thus, if variations in size are taken into consideration, the basal metabolism of our albino rats was well within the range of the commonly accepted values for albino rats.

With our two wild rats for which basal data are available the heat production was 792 and 828 calories per square meter, respectively. These values are higher than those reported for the albinos but measurably lower than the two averages of 933 and 1013 calories noted with wild rats by Benedict and Petřík.

Huddling. The fact that the huddling of a group of animals reduces the surface area exposed to the surrounding temperature raises the question as to whether animals massed together reduce heat production. Data as yet published only in abstract (Benedict and Fox, 1933) have shown that with the young albino rat (40 to 50 days old, weighing about 60 gm.) the basal metabolism at thermic neutrality was the same whether the animals were huddling or separated. This finding is in accord with observations on sheep (Benedict and Ritzman, 1931). It may be argued, however, that although huddling plays no rôle at thermic neutrality, it might have an effect at lower temperatures when chemical heat regulation takes place. For this reason we made observations on a group of four albino rats (average weight 165 gm.), 20 hours after food, at a temperature of about 22°C. The rats had been living at this same temperature prior to the tests. On some days they were allowed to huddle in the chamber and on other days were separated from each other by partitions. When separated, they were often active for from 50 to 80 per cent of the experimental period. When huddling, they were usually much quieter. In the following averages, representing the 24-hour heat production per square meter of body surface, we have included only those observations made when the rats were quiet for 75 per cent or more of the period of measurement.

Huddling	*Separated*
817 cals.	680 cals.
734 cals.	781 cals.
828 cals.	
802 cals.	
768 cals.	

In nearly all cases the activity as well as the heat production was higher at 22° than that commonly found under basal conditions (28°). With these rats the picture, although irregular, is certainly in support of the conception that huddling, even at a temperature below thermic neutrality, has no significant effect upon the heat production.

Skin temperature of the rat's tail. The rôle played by the tail and the ears of an animal as areas for heat loss has been frequently considered (Przibram, 1923). We made a few measurements with a delicate thermojunction of the skin temperatures of the tails of four of our albino rats, for comparison with the environmental temperature. At room temperatures of 24° to 26°C. the skin temperatures averaged less than half a degree above the environmental temperature. Hence the significance

of the tail as an area for heat loss is by no means as great as was formerly thought.

Effects of fasting. Length of life. Of our eight young albino rats fasting at 28°, one survived for 24 days but the majority did not last 16 or 17 days as did three of the Horst rats which were older (Horst, Mendel and Benedict, 1930). Indeed one lived only 7 days. Hence the picture as a whole suggests that younger rats have somewhat less resistance to fasting than do older rats. The ages of our wild rats are not known, but since most of them were small (only three out of nine weighed over 200 grams and five weighed 136 grams or under), they probably were young. All of our wild rats succumbed much more rapidly to fasting than did the albinos, only one lasting 9 days and the others dying on or before the sixth day. In general it would appear as if the wild rats had somewhat less resistance to fasting than the laboratory-bred rats, but it is possible that the factor of size or age may play a rôle here.

Losses in body weight. With our albino rats the percentage losses in weight, based upon the weights at the beginning and end of each fasting period, ranged from 29.4 to 57.1 (average, 39.0 per cent) and with our wild rats from 29.5 to 43.2 (average, 35.1 per cent). (See tables 1 and 2.) Since both groups included a large proportion of young or at least not adult rats, the comparison here is probably justifiable. The conclusion is that the wild rat not only succumbs more rapidly to fasting than does the albino rat but also before it has lost as great a percentage of body weight.

Feces. A rough index of the rate of passage of food through the intestinal tract is obtained from a record of the feces pellets passed daily. All rats in both groups passed from 12 to 15 pellets on the first day of fasting. Thereafter, for one or two days, the albinos usually passed only two pellets per day, and after about the third or fourth day no feces were passed. The wild rats had a much slower discharge, for two of them passed 12 to 14 pellets even after three or four days of fasting and in one instance there were 22 pellets on the fourth day. After this, however, rarely were any feces passed. In one instance (rat 4) even on the 19th and 21st days of fasting, one and two pellets, respectively, were passed. No instances of coprophagy were noted.

Heat production during fasting. Heat production per 200 grams of body weight. The tremendous changes in body weight, along with the changes in metabolism of these fasting rats, make direct comparisons between the total heat production at the beginning and end of fasting difficult. At least it can be said that the total heat production at the end was frequently only about one-third that at the beginning. The body weights also decreased, but never to as low as one-third the initial weight. Hence comparisons of the metabolism of these fasting rats are more directly made with reference to the body weights. (See tables 1 and 2.) Per 200 grams

TABLE 1

Metabolism of laboratory-bred rats during prolonged fasting*

(June 7–July 31, 1930; living at 28°; measured at 28°C.)

RAT NUMBER AND PER CENT LOSS IN WEIGHT**	BODY WEIGHT	DAYS AFTER FOOD	R.Q.	HEAT PRODUCED PER 24 HOURS			NITROGEN EXCRETED PER 100 GRAMS PER HOUR	CALORIES FROM PROTEIN
				Total	Per 200 grams	Per sq.m.		
	grams			*cal.*	*cal.*	*cal.*	*mgm.*	*per cent*
Males								
No. 1	137	½	0.75	14.7	21.5	607		
31.3 per cent	112	4	0.73	11.8	21.1	560	4.36	26
	106	6	0.74	10.1	19.0	493	4.60	31
	98	7†	0.82	7.1	14.4	365	4.98	44
No. 2	116	1	0.73	13.7	23.7	636		
29.4 per cent	103	3	0.71	12.0	23.2	598		
	85	7†	0.81	7.7	18.0	436		
No. 3	121	1	0.73	17.4	28.9	782	2.93	13
43.4 per cent	115	2	0.72	16.1	28.0	750	3.08	14
	107	4	0.72	13.9	26.0	678	2.13	10
	100	6	0.72	11.3	22.5	575	2.28	13
	96	7	0.73	11.7	24.4	611	2.71	14
	93	8	0.74	11.4	24.5	610	2.80	15
	87	9	0.75	11.4	26.2	637	3.47	17
	84	10	0.76	7.8	18.5	444	3.60	25
	80	11‡	0.80	6.0	15.0	355	6.10	52
Females								
No. 4	142	1	0.71	18.6	26.2	750	4.75	23
57.1 per cent	138	2	0.72	17.2	24.9	706	4.88	25
	133	3	0.70	17.5	26.3	737	2.08	10
	131	4	0.71	14.8	22.6	630	2.11	12
	116	8	0.73	14.3	24.7	664	1.58	8
	110	10	0.72	13.8	25.1	660	1.31	7
	107	11	0.72	14.4	27.0	704	1.58	7
	101	14	0.71	9.8	19.4	496	1.67	11
	95	16	0.71	10.4	21.9	550	1.72	10
	90	18	0.75	8.8	19.6	481	1.81	12
	89	19	0.75	9.1	20.5	503	1.83	11
	80	21	0.75	9.7	24.2	574	1.93	10
	73	23	0.76	8.8	24.2	555	3.45	18
	68	24‡	0.82	5.6	16.5	368	3.71	29

* Rats from colony of Prof. W. E. Castle.

** Per cent loss in weight is based upon last weight before death and initial weight immediately after removal of food, and can not be calculated from the weights shown in this table.

† Died within 24 hours after experiment.

‡ Died about 6 hours after experiment.

TABLE 1—*Concluded*

RAT NUMBER AND PER CENT LOSS IN WEIGHT**	BODY WEIGHT	DAYS AFTER FOOD	R.Q.	HEAT PRODUCED PER 24 HOURS			NITROGEN EXCRETED PER 100 GRAMS PER HOUR	CALORIES FROM PROTEIN
				Total	Per 200 grams	Per sq.m.		
	grams			*cal.*	*cal.*	*cal.*	*mgm.*	*per cent*
Females—Concluded	92	¾	0.72	12.3	26.8	666		
No. 5	82	2	0.73	10.5	25.6	609	3.65	18
36.2 per cent	62	7†	0.80	6.3	20.3	441	3.60	22
No. 6	107	2	0.72	12.1	22.5	588	2.87	16
42.5 per cent	90	7	0.73	10.0	22.2	547	2.76	16
	87	8	0.73	10.0	22.9	557	2.85	16
	86	9	0.73	7.9	18.5	448	2.88	20
	83	10	0.74	7.5	18.2	435	3.29	23
	80	11	0.77	6.6	16.4	388	3.41	26
	75	12†	0.79	5.2	13.9	321	6.11	56
No. 7	126	¾	0.72	16.1	25.6	705		
33.8 per cent	126	1	0.72	16.4	26.0	714	2.60	13
	99	6	0.75	9.9	20.0	508	5.60	36
	92	7¶	0.80	5.7	12.4	308	6.02	62
No. 8	134	2	0.70	15.9	23.8	669		
38.5 per cent	121	4	0.72	13.3	22.0	598	2.92	17
	107	7§	0.73	9.4	17.5	457	3.30	24

¶ Died a few minutes after experiment.
§ Died about 57 hours after experiment.

of body weight (the average weight of the adult rat) the heat production of our young albino rats decreased pronouncedly throughout the fast except with rat 4 on the 21st and 23d days, when there was an increase. This decrease is in striking contrast to the uniform heat production per unit of weight noted with the more adult fasting female rats studied by Horst, Mendel, and Benedict (1930). With our wild rats the data on days intermediate between the first and the last day of the fast are meager, but in general there is again a tendency to uniformity in the heat production per unit of weight up until nearly the end of the fast with the adult animals and a rapidly decreasing heat production per unit of weight with the younger animals.

Heat production per square meter of body surface. On the basis of surface area ($S = 9.1 \times w^{2/3}$) the metabolism of our albino rats likewise decreased continually with the progress of the fast, and on the last day extraordinarily low values were found. According to an earlier publication (Benedict, Horst and Mendel, 1932) a large male rat on the 29th and the 43rd day of fasting had an extremely low heat production of 377 and 381 calories,

TABLE 2

Metabolism of wild rats during prolonged fasting

(July 21–30, 1930; living at 28°; measured at 28°C.)

RAT NUMBER AND PER CENT LOSS IN WEIGHT*	BODY WEIGHT	DAYS AFTER FOOD	R.Q.	HEAT PRODUCED PER 24 HOURS			NITROGEN EXCRETED PER 100 GRAMS PER HOUR	CALORIES FROM PROTEIN
				Total	Per 200 grams	Per sq.m.		
	grams			*cal.*	*cal.*	*cal.*	*mgm.*	*per cent*
Males								
No. 9	136	4**	0.72	14.2	20.9	591		
31.0 per cent								
No. 10	175	4	0.73	19.6	22.4	686	6.97	40
35.3 per cent	157	5	0.78	17.7	22.6	669	10.01	56
	141	6†	0.80	11.7	16.5	472	11.14	85
No. 11	222	1	0.73	26.5	23.9	792		
34.6 per cent	185	4	0.80	21.1	22.8	715	4.90	27
	177	5**	0.80	17.3	19.6	603	5.12	33
No. 12	80	3**	0.72	12.7	31.6	749		
33.7 per cent								
Females								
No. 13	78	3**	0.72	11.2	28.8	675		
33.3 per cent								
No. 14	129	4	0.71	19.6	30.4	845		
43.2 per cent	125	5	0.74	15.7	25.1	687	4.10	21
	119	6	0.71	13.6	22.9	618	4.31	24
	116	7	0.77	10.6	18.3	491	4.42	31
	96	9‡	0.79	6.5	13.5	340		
No. 15	321	1	0.73	35.3	22.0	828	5.48	32
29.5 per cent	241	5‡	0.81	19.1	15.9	544	10.02	85
No. 16	107	5	0.78	10.6	19.7	516	8.12	52
38.1 per cent	103	6¶	0.80	6.3	12.2	315	8.44	88
No. 17	190	5	0.78	20.3	21.4	675	9.39	56
37.3 per cent	175	6§	0.81	16.9	19.3	592		

* Per cent loss in weight is based upon last weight before death and initial weight immediately after removal of food, and can not be calculated from the weights shown in this table.

** Died some time within 42 hours following experiment.

† Died on same day as experiment.

‡ Died within 12 hours after experiment.

¶ Died about 5 hours after experiment

§ Died at unknown time after experiment.

respectively, but continued to live under fasting conditions for nearly two weeks longer, when it was killed. In the case of the rats listed in table 1, however, the low values found on the last day occurred within a short time before death. Thus with five of our albino rats six values of 388 calories or under were noted at the end of the fasts and in only one case (rat 6; heat production on the 11th day, 388 calories; death within 48 hours thereafter) was a low value observed any appreciable length of time before death. No correlation between the lowest level of metabolism and the initial weight or length of fast is to be observed. The lowest value (308 calories) was found on the 7th day with rat 7, but at least three other rats survived longer than this with a somewhat higher metabolism. If values of 400 calories or under are left out of consideration, as representing the effect of a moribund condition,[1] the next lowest values with these albino rats range from 555 calories with rat 4 on the 23d day to 435 and 436 calories with rats 6 and 2 on the 10th and 7th days. With the wild rats the heat production showed a decrease in those instances when more than one measurement was made, and values below 350 calories were noted in two cases (nos. 14 and 16). These two rats had lost the most in weight of any of the wild rats. Of the wild rats studied by Petřík, three withstood fasting for 8 days or for a longer time than most of our wild rats, and yet at the end of 8 days their metabolism was not strikingly low, being 603, 613, and 698 calories, respectively. However, our wild rats were younger than the Petřík rats, and it appears that the youthful organism does not withstand fasting as well as the adult organism. In general the values show a higher metabolism prior to the last day of measurement than with the albinos, which confirms the belief that the metabolism of the wild rat under the same conditions is higher than that of the albino rat.

Respiratory quotient. With our albino and wild rats the observations made under basal conditions, 17 to 50 hours after removal of food, showed respiratory quotients averaging 0.73. In no case were quotients noted of 0.80 or higher, such as have frequently been reported by other investigators for rats after about 15 to 24 hours of fasting. In unpublished experiments by Dr. Thorne M. Carpenter of the Nutrition Laboratory the highest respiratory quotient noted in 39 measurements with rats under basal conditions (15 to 17 hours after food) was 0.73, and this occurred in only four instances. We believe the closed-circuit method can not be relied upon to give accurate respiratory quotients and that when the respiratory quotient is to be used either as an index of carbohydrate combustion in the body or as an index of a greatly increased protein katabolism, it must be accurately determined by gas analysis.

[1] With three moribund rats studied by Benedict and MacLeod (1929) the heat production just before death amounted to 352, 197, and 189 calories per square meter of body surface.

With nearly every one of our rats, both the albino and the wild rats, the respiratory quotients at the end of fasting increased from a general fasting level of about 0.72 to values around 0.80. With rat 4, which fasted for the longest period, the rise in the respiratory quotient did not begin until the 18th day. On this day the quotient averaged 0.75 and thereafter it increased to a maximum of 0.82 on the 24th day. With the other rats, which survived for shorter periods, the rise in the quotient began about the sixth or the seventh day. These high quotients suggest that the rat was drawing heavily, if not exclusively, upon protein at the terminal stage of the fast.

Urinary nitrogen excretion. The urinary nitrogen output of our rats, measured simultaneously with the respiratory exchange, can be considered only as approximate for the given periods of time, since the analyses were based upon collections of urine spontaneously voided and not sharply separated with regard to time by catheterization or pressure. The nitrogen excretion per 100 grams of body weight per hour decreased as the fasting progressed, but in nearly every case rose pronouncedly shortly before the death of the rat. Cori and Cori (1926) report that the rat between the 24th and the 48th hours of fasting has a nitrogen excretion amounting to about 3 mgm. per 100 grams of body weight per hour. With relatively few of our rats was the urine collected for precisely this period of fasting, but in those instances with the albino rats when collections were made after one and two days of fasting the nitrogen excretion per 100 grams was not far from 3 mgm. On the contrary, with the one wild rat whose urine was collected after 24 hours of fasting the excretion was nearly twice as high. Furthermore, in the later stages of fasting (leaving out of consideration for the moment the last day) this relatively higher nitrogen excretion persisted with the wild rats. This implies that the wild rat has a considerably greater protein katabolism than the albino rat, even before the terminal stages of fasting.

The fact that with most of these rats there was an increase in the respiratory quotient and in the nitrogen excretion toward the end of the fast suggests that the fat depots had been in large part depleted and the rat was drawing heavily upon body protein. In accordance with the ideas of Rubner concerning the specific dynamic action of protein, one might expect that as a result of the increased protein katabolism at the end of fasting there would be an increase in the total metabolism. Such an increase in total metabolism was noted when phlorhizin was administered to a fasting dog (Rubner, 1902). One might argue, therefore, that when during fasting alone the katabolism becomes exclusively a protein combustion, there would be an increased stimulus to metabolism. But certainly this does not appear to be the case with the rat, for on those days when the nitrogen output and the respiratory quotients were the highest, the 24-hour heat production was the lowest. Indeed, prior to the last few measure-

ments there was rarely a hint of an increase in the metabolism in any of the factors measured. The lack of increase in the total metabolism at the terminal stage of fasting is a resultant of two contending forces, one the dying condition of the animal which would tend to lower the metabolism, and the other the increased protein combustion which would tend to increase the metabolism. It would appear, however, as if the entire metabolic process in the case of these fasting rats was not to be compared for a moment with the metabolism following the feeding of protein or phlorizin poisoning.

Calories from protein. The non-protein respiratory quotients of our rats have not been calculated, for it is debatable whether the standard factors commonly used to compute such quotients, based upon observations made at relatively early stages of fasting, are applicable to the terminal stages of fasting. From the nitrogen excretion and the total metabolism we have computed the total calories derived from protein, as shown in the last column of tables 1 and 2. Here it is evident that with prolonged fasting, particularly in the terminal stages, there is a greatly increased protein combustion. Indeed, with three of the wild rats the protein furnished 85 per cent of the total calories.

SUMMARY

Huddling at 22° had no effect upon the heat production of rats. At room temperatures of 24° to 26°C. the thermal gradient between the skin temperature of the rat's tail and the temperature of the environment was small.

Young rats withstood fasting for a shorter time than older rats. The wild rats not only succumbed more rapidly than the albinos but before they had lost as great a percentage of body weight. The rate of passage of food through the intestinal tract was slower with the fasting wild rats.

Confirmation of the results of a previous investigation is given in the finding that the wild rat has a measurably higher basal metabolism than the albino rat. Even toward the final stages of fasting the wild rat's metabolism is likewise higher than that of the albino. In all cases fasting (at 28°C.) caused a marked decrease in the total heat production and in the heat production per unit of body weight and of body surface. The total metabolism just before death was often only about one-third the initial level. With moribund rats values between 300 and 400 calories per square meter of body surface per 24 hours were noted.

Under basal conditions the respiratory quotient averaged 0.73 with both races of rats. Towards the end of fasting the quotients increased to about 0.80. This increase did not begin until the 18th day of fasting with one albino rat that survived 24 days, but with the other rats of both races, which lived for shorter periods, the rise began on the 6th or 7th day.

These high quotients show that the rat draws heavily upon body protein at the terminal stages of fasting.

The urinary nitrogen excretion per 100 grams of body weight per hour decreased as the fasting progressed, but rose again pronouncedly shortly before death. It was greater with the wild than with the albino rat, both in the early and final stages of fasting. The moribund rat derives the larger proportion of its total heat production from the combustion of protein.

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SOME PROPERTIES OF THE CORD POTENTIALS EVOKED BY A SINGLE AFFERENT VOLLEY

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When a lead is made from the exposed spinal cord of the cat and a dorsal root is stimulated with a single induction shock, there follow electrical potential changes which last for some time. These have a complexity which varies with the conditions obtaining in the experiment. Gasser and Graham described some of the simpler potential pictures to be observed but left the more complicated ones for later analysis. Further observations on these potentials are recorded in this paper. The experiments are exploratory in nature. Cats in various stages of ether narcosis are described along with others in the acute decerebrate or spinal state, after a lapse of time sufficient for elimination of the anesthetic. The methods of leading are varied, involving the double brush-lead from the dorsum of the cord used by Gasser and Graham, silver electrodes placed in different paired positions on the cord, and the needle electrodes of Adrian and Bronk. Some comparisons are made between the cord potentials and the discharge into the sciatic nerve.

Leads from dorsum of cord. The general experimental procedure was similar to that previously described. After exposure of the spinal cord and opening of the dura stimulating electrodes were placed on the seventh lumbar or first sacral root. The latter were silver wires inserted into a small slotted hard rubber tube. After the nerve had been placed in the electrode it could be returned to its natural position and the electrode covered with a bit of cotton soaked in Ringer's solution. Irritability was thereby conserved to the root throughout the course of an experiment, which might be 6 hours or longer. No difficulty was encountered in preventing escape of stimulation from one root to the next.

Camel's hair brushes served as electrodes. A brush was impaled from the quill side with a silver wire; and the quill, when filled with Ringer's solution, served as a reservoir keeping both the brush and the surface of the cord moist. With a holder attached to an adjustable Palmer stand the electrode could be brought into the desired approximation to the cord.

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In the standard arrangement one lead placed a few millimeters above the uppermost strand of the root was connected to the ground; the other, several centimeters above it was connected to the grid. The position of the second lead was not of great importance; but the size, and to some extent the form of the potential derived, depended on the position of the first lead. The records were made in such a way that an upward deflection meant negativity of the lead nearest the root.

The magnitude of the potential is greatest at the root level and falls off rapidly above and below it. For purposes of illustration some point on the complex contour must be selected and the one chosen is the first crest of the negative intermediary potential, as it is easily measured, appears in all records, and, except in very irritable preparations, is the highest one.

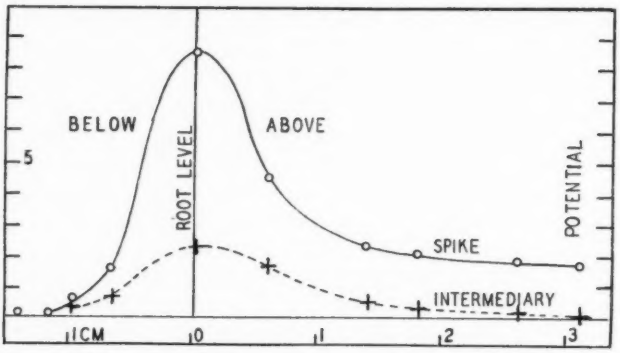

Fig. 1. Magnitude of the intramedullary spikes and the corresponding negative intermediary potentials plotted at various levels in the cord.

The curve yielded in a typical experiment is shown in figure 1. When compared with the curve of the heights of the intramedullary spikes shown in the same figure a general similarity is apparent. The only difference is that the latter continues above the point where the former shows no further detectable potential. The decrease in the height of the spike has been interpreted as due to termination of first order neurones and a decrease in the number of their collaterals. Connections with second order neurones would be fewer and from this it would naturally follow that the negative intermediary potential should decrease. In the method of leading the localization is not exact and the error is such as to show a slower falling off than actually occurs. However, several considerations indicate that the curves are not far from the truth. Localization is good enough to permit measurement of the velocity of conduction of the intramedullary spike. Then, too, if the curve were merely the effect of distance from a

potential at the root level it should be approximately symmetrical above and below it; and, finally, exploration with a needle electrode, for which the localizing power is better, yields data supporting those obtained with the standard lead.

The basic form of the cord potential, such as is seen in narcosis, shows a period in which the proximal electrode is negative followed by one in which it is positive. Variation consists in the size of the positive wave and the complexity of the negative potentials which develop in more irritable cords. The properties of the simple negative wave have been previously described (6); to this description may now be added those of the positive wave. To avoid confusion it should be repeated that by the designation "positive wave" no further implication is included than that in leads from the dorsum of the cord the electrode proximal to the root is positive to the distal one.

Strength of stimulus and the positive wave. The positive wave is confined mainly to neurones in connection with low threshold fibers in the root. It appears when a threshold induction shock is applied to the root, but as the strength of the shock is increased the wave soon reaches a maximum and further changes in potential are confined to the negative components. The momentary value of the potential is determined by an algebraic summation of constituents recording in the two directions and the transition point from negative to positive can be considered as marking neither the ending of one process nor the beginning of another. Potentials which by themselves would record as positive can be shown to exist in the presence of a negative sum; and negative waves produced in the presence of a large positive wave may fail to raise their heads above the zero level. Illustrations of these combinations are seen in the following experiments. Figure 2*a* shows the typical picture seen in deep narcosis: a simple negative wave followed by a positive. Figure 2*b* shows the effect of elimination of ether. An additional negative wave appears which encroaches on the early part of the existing positive wave, giving it apparently a later start. The part of the positive wave thus covered up can again be revealed by evoking the response during a period of subnormal responsiveness occasioned by activity persisting from a previous afferent volley. The later negativity then falls away (fig. 2, *c* and *d*). In very active preparations a positive wave may be entirely obliterated by negative waves (fig. 4*b*), but when so obliterated it can again be completely uncovered by locating the whole process in an unresponsive period (see fig. 3 of the following paper). Figure 3 shows the effect of a deepening of the positive wave which occurred during the course of an experiment. The later portion of the negative wave, as seen in part *a*, when written on the deepened positive wave as a base line (part *b*), lies below the level of zero potential.

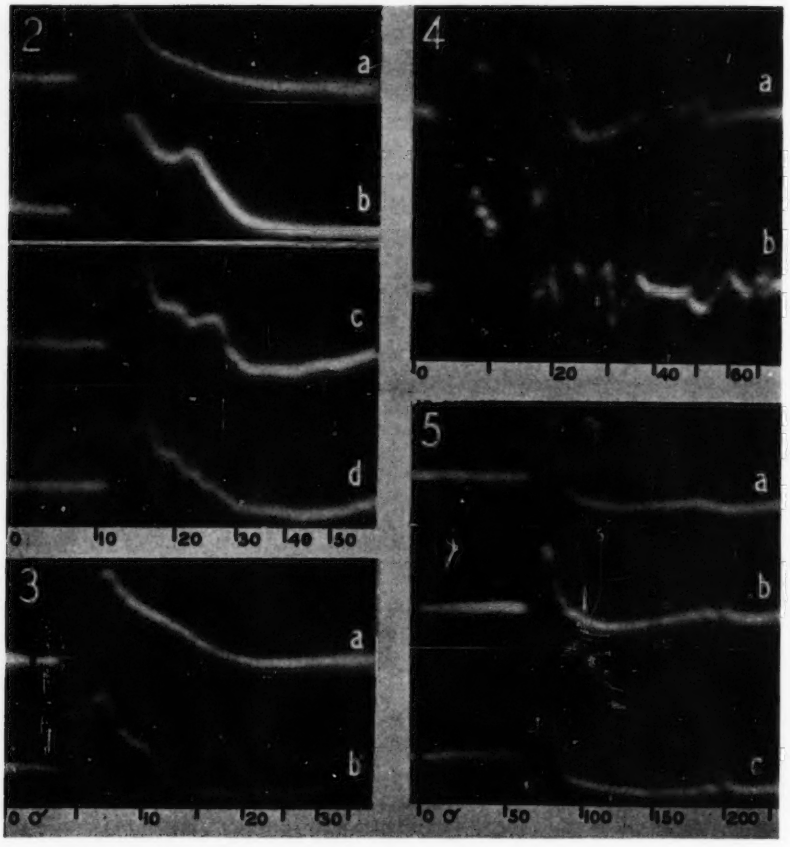

Fig. 2. Form of cord potentials. *a*, deep ether narcosis. *b*, light ether narcosis in the same preparation (2/6/33). *c*, spinal preparation; no ether for 3 hours. Response to a single afferent volley. *d*, modification of the response to a similar afferent volley produced by a like volley 231σ earlier (9/7/33).

Fig. 3. Effect of the size of the positive wave on the apparent duration of the negative. *a*, no definite positive wave developed. Long period of negativity; ether anesthesia. *b*, response 3 hours later, 30 minutes after decerebration. Positive wave present; period of negativity shorter (3/23/33).

Fig. 4. *a*, decerebrate preparation 2 hours after cessation of anesthesia. *b*, spinal preparation of the same cat. Record made 10 minutes after transection of the cord in the lower thoracic region (10/10/33).

Fig. 5. Effect of simultaneous stimulation of the 7th lumbar and 1st sacral roots. *a*, stimulation of 7th lumbar. *b*, stimulation of 1st sacral. *c*, stimulation of both together (6/16/33). Ether anesthesia.

Convergence of the positive wave. It was previously shown that the negative portion of the potential, as elicited by the simultaneous stimulation of two roots, as L_7 and S_1, was regularly far less than the sum of the individual negative waves derived from each one alone. This could only mean that many of the neurones invaded by the impulses from one root were also accessible to those from the other. A similar analysis remained to be made for the positive wave.

Four successful experiments were concordant in showing that the positively recording potential likewise is generated beyond the point at which the pathways from the two roots overlap. Not only did simultaneous stimulation of two roots produce a positive wave much less than the sum of the isolated controls (fig. 5), but stimulation of one root *after* the other produced a reaction with a deficit of positive potential in the second response (fig. 6). A comparison of the amount of overlap indicated for the negative and positive potentials showed it to be similar. A large deficit in one potential went with a large one in the other, and small differences could not be considered as significant because of the measurement of height instead of area and because all the constituents contributing to the negative wave do not have the same amount of positivity attached to them. The similar position of origin of the two potentials with respect to the point of convergence strongly suggests that they are produced in the same neurones.

The positive potential as affected by the position of the leads. As a possible explanation of the positive potential it was previously suggested (6) that in some dorso-ventrally oriented structure negativity appearing at the dorsal end might migrate ventrally and persist in the latter position after it had died out in the former. In order to test this possibility and to gain more information concerning the distribution of potentials within the cord other derivations were added to the standard lead. After placing the additional electrodes in position, provision was made for the selection of pairs in rapid succession. For leads from the ventral side of the cord silver lamellae about 4 mm. in diameter, insulated on one side and connected to fine insulated wires, were inserted between the cord and the ventral dura. The arrangement of the electrodes is shown in figure 7; and the potentials corresponding to the several leads are shown semi-diagrammatically in the second column of the same figure. A deflection upward means that the first mentioned electrode, as *A* in the *A-B* lead, is negative. Lead *A-B*, which is standard, gives the usual picture. A shift to *A-C* gives a result which is quite similar. The lead *C-D*, which is the ventral lead symmetrical to the dorsal *A-B* lead, may give a picture of the same form but of the opposite sign to the latter. It is smaller but the size is of no significance on account of the shunting by the spinal fluid and surrounding tissues, which does not obtain in the dorsal lead. An alternative finding of

equal probability is a monophasic potential; the potential starts out as the reverse of the negative portion of the dorsal lead but, instead of crossing the line of zero potential, it ends from the side on which it started. The lead *B-D* shows that the potential developed is very much smaller, 2 or 3 cm. above the root. Usually *B* remains negative throughout its course, but occasionally a positive wave develops. It is then like the *A-C* lead only smaller.

In the first phase, corresponding to the duration of the negative wave of the standard lead, the potentials are as indicated by the arrows in figure

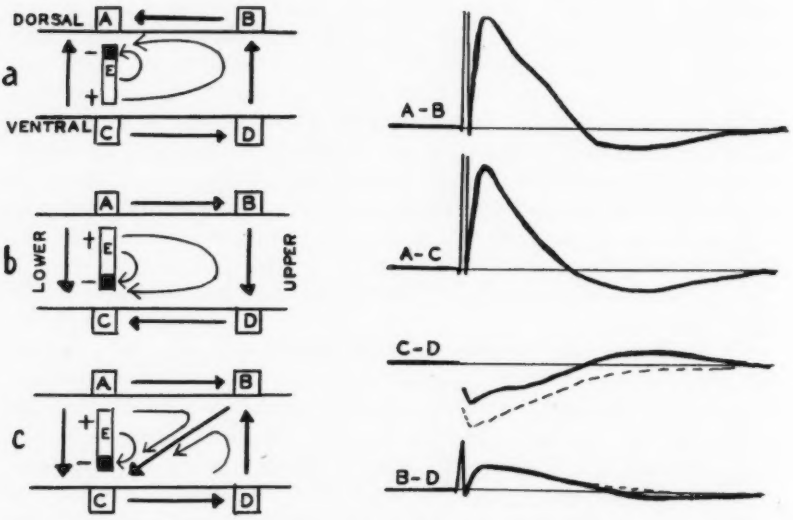

Fig. 7. The effect of the position of the leads on the form of the potential. *A*, *B*, *C*, *D*, electrodes in position. Heads of arrows at negative end. Heavy arrows with solid heads show the potential relationship between the electrodes as actually determined; light arrows, theoretical eddy currents. *E*, hypothetical unit generating potential.

7*a*. A sufficient explanation for such an arrangement would be the production of the potentials in a set of generating units, with their maximum negativity at the dorsal end. In the second phase, corresponding to the positive wave of the standard lead, two arrangements appear. When the potential is diphasic in the four leads all the arrows would be reversed, as in figure 7*b*, and the theoretical eddy currents would be in simple reversal from the initial direction; but when the potential is monophasic in the *C-D* and *B-D* leads the eddy currents are only partly reversed. Because *B* is positive to *C* in the *B-C* lead the eddy currents may be drawn in re-

versal in the triangle *ABC* (fig. 7*c*); but on account of insufficient relative negativity in the region of *D*, they must be represented as running in the original direction in the triangle *CDB*.

As the information obtained with the various external leads was not incompatible with a dorso-ventral shift of negativity, the possibility was tested further in some experiments performed with the concentric needle electrodes described by Adrian and Bronk. On connecting the needle to the ground and the core to the grid a potential was obtained resembling the standard lead, the needle being negative to the core at the same time that *A* is negative to *B*. As the needle was pushed ventralward from the dorsal surface of the cord in the direction *A-C* (fig. 7) the potentials recorded increased in size until the tip reached the center of the cord; beyond this point they decreased. No constant change in form appeared. In other words, there was no evidence that negativity developed at a later time in the ventral region in such a way as to account for the late positivity of *A* to *B* in the standard lead. Unfortunately, however, tests of the localizing power of concentric electrodes proved the localization to be inadequate to make the point conclusive. Localization with double-wire needle electrodes was found to be still less exact on account of the poor ground lead provided in this form in comparison with the small leads to the ground from the animal and apparatus connected to it. Therefore the needle technique was not followed further.

The change from negative to positive can readily be explained without a shift in negativity from the dorsal to the ventral side of the cord, as a like effect would be produced in an external lead by a shift of dominant negativity in any element, no matter what its length or position, provided that the projection of the shift move ventrally along the dorso-ventral diameter or centrally along the longitudinal axis.

A potential difference between the parts of a neurone must occur; otherwise there would be no external potential sign. Because of the short length of the neurones in the gray matter of the cord, conduction must be subordinate in the production of potential differences and the latter must largely be due to intensity or duration. A neurone is made up of strands of protoplasm of very different diameters, varying from the thick perikaryon to the minute terminal *boutons*, and in analogy with peripheral nerve the expectation would be that the potential in the thin strands would outlast that in the thick ones (the size of the potential cannot be inferred from the nerve-analogy, as the small potentials obtained from fine nerve fibers are sufficiently accounted for on the basis of the high internal resistance of such fibers). The cell body should develop its negativity faster and lose it sooner; thus there would be gradients toward the cell initially and away from it later, and the sign of the potential in the external effect would depend upon the arrangement and size of the branches. No

essential difference in the argument would occur if the potentials are considered to be after-potentials. Such a view has little to support it, but it cannot be excluded by the known facts. It would permit assumption of actual positivity with respect to the resting state and this positivity would give a positive deflection in the records if dominant at the dorsal pole.

The negative waves. Even in the deepest narcosis there remains in the cord response a negative wave having a mean duration of 10σ (6), provided that the circulation has not been impaired. If the narcosis be allowed to become less deep a second negative wave appears on the falling phase of the first. It may have a definite crest (figs. 2*b* and 8*b*) or merely appear as an excrescence on the basic curve (fig. 3, *a* and *b*). Still lighter narcosis leads to the appearance of signs of a third and a fourth constituent. At this point the reflexes become too active for steadiness in recording and decerebration or decerebration followed by transection of the cord in the lower thoracic region must be employed. The condition of the animal after the operation is very important in determining the type of picture obtained. When the cord is in poor condition, even after a long period without anesthesia, the response to stimulation is not very different from what it is in narcosis. When the cord is in better condition constituent negative crests become prominent (figs. 2*c* and 9*b*), and in still more irritable preparations the picture is extremely complex. The anesthesia variable does not permit one to ascertain the effect of decerebration on the cord potentials and the change from decerebrate to spinal is rendered somewhat obscure by the fortuitous changes in condition. However, if the transection of the cord be made after a long period of freedom from ether and be attended by but a small amount of shock, in the period immediately succeeding the transection the number and distinctness of the waves tends to become greater (compare *a* and *b* in fig. 4). The change is of interest because of the well-known alteration in the flexor reflex which follows the transition from the decerebrate to the spinal state; but its description is given here more to illustrate the experimental variations which are to be encountered in the cord than to typify the states themselves.

The group of waves following the intramedullary spike may be called for descriptive purposes the "first negative complex." The constituent waves have a duration of an order of 3 to 5σ; which, considering the probable asynchronism, means that the potentials at their source are not very different in length from the spikes of peripheral nerve. The successive additions of new waves as the cord becomes more irritable are very simply explained by the assumption that the irritability of more aggregates of neurones is rising above threshold, thereby adding their potentials (and possibly those of tracts coming from them) to the total. In the less

irritable cords only the neurones most directly in line with the root fibers would respond to an afferent volley, as they would be the only ones having a sufficiently dense population of active terminals on their surfaces for excitation to be effected. In the more irritable cords the neurone thresholds would be lower and neurones receiving fewer endings could be excited. These in turn would add their endings to the total, thereby permitting a still greater invasion of the nervous system by the impulses.

The crests of the added waves are not always in the negative direction; in fact, it is often impossible to decide whether the alteration from the smooth contour seen in narcosis is a spike-like addition or a spike-like depression. That some of the crests are in the positive direction is undoubted, because they fall below the line of zero potential; and one wave which regularly appears in the best preparations at 7σ (fig. 4*b*) is so large that it is possible to identify the fact that it is diphasic in form.

Period of discharge into the sciatic nerve. As the reduced reflex time of the flexor reflex is around 3σ it is desirable to know what is going on in the sciatic nerve during the first negative period. This was studied mostly under light ether, and records from a sample experiment are shown in figure 8. The upper record is from the sciatic nerve. It shows, in view of the amplification used, that but a small part of the motor nerve fibers was active. Under the reduced temperature of the nerve a motor axon-spike would last about 1σ. The potential is not yet ended at 21σ, therefore the temporal dispersion is great. After discounting the temporal dispersion of conduction from the cord to the electrode, which is small because motor fibers are a relatively homogeneous group, it follows that the cord was sending out impulses during most of this period. A direct comparison with the corresponding cord potential, reproduced in the lower part of the figure, shows the period of discharge into the sciatic nerve to correspond very well with the period of negative potential. No positive potential is visible in this instance; but, in cords showing a positive wave, the sciatic nerve would receive impulses up to the crest of the wave, showing again that potentials having the same significance as the earlier negativity must exist even after the algebraic sum of the potentials is on the positive side of zero.

An attempt is made in figure 8 to correlate the two potentials as to time. The discharge into the nerve cannot start until after the reduced reflex time. Gasser and Graham showed that, when a flexor reflex first appears, a small disturbance of the contour of the cord potential occurs at the point marked by the arrow in the figure, a point which also corresponds to the reduced reflex time as it has been determined by a number of observers (4) (5) (7). We find the disturbance apparently to have the character of a thin crescentic indentation. Its duration is marked by the first thickening of the base line in the figure and corresponds to the first big discharge into

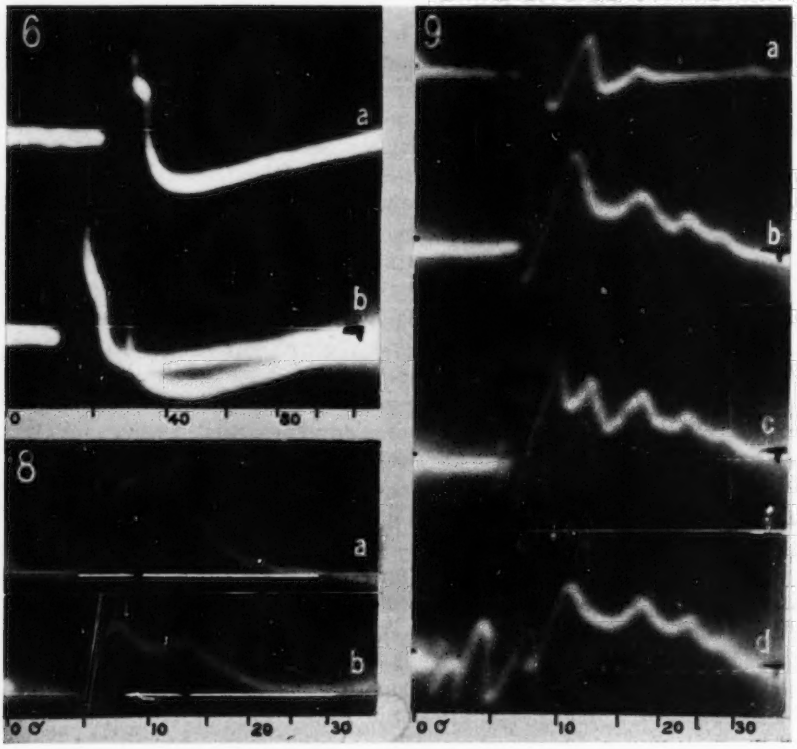

Fig. 6. Convergence and the positive wave. *a*, cord response produced by a volley from the 7th lumbar root. *b*, print of two records superimposed. One record is the response to a volley from the 1st sacral root; the other is a similar response, followed at an interval of 18σ by a response to L_7. For the first 18σ the records are alike (response to S_1); then the potential added by L_7 appears. If there had been no convergence the total positivity should have been that of S_1 plus the amount L_7 shows in part *a* (10/4/33).

Fig. 8. Comparison of the response in the sciatic nerve: *a*, with that in the spinal cord; *b*, when a dorsal root is stimulated. Arrow marks reduced reflex time. Experiment performed under light ether anesthesia (9/30/33).

Fig. 9. The effect of antidromic impulses in motor fibers on the response to an afferent volley from a dorsal root. Stimuli applied to the sciatic nerve after all dorsal roots had been cut. Spinal preparation. *a*, effect of antidromic impulses only; *b*, response to an afferent volley; *c*, antidromic and afferent volleys arriving at the cord simultaneously. The result is the algebraic sum of *a* and *b*. *d*, antidromic volley arriving ahead of afferent volley. The effect of the isolated antidromic volley as it would appear at this position in the line is drawn in for reference (9/13/33).

the sciatic nerve (similarly marked). The remainder of the discharge into the nerve would then correspond to a second and longer crescent in the cord. After-discharge has not been studied.

In the preceding paragraph the potential assigned to activity of motor neurones was small. It is justifiable to inquire whether any of the larger constituents of the initial complex, especially as they are seen in spinal preparations, can be so assigned. To answer this question experiments were performed in which antidromic impulses from single induction shocks were brought to the cord over motor fibers according to the method of Denny-Brown. When these were pitted against impulses coming in the normal direction, so as to meet the latter at various times in their course, all that was ever obtained (standard lead) was an algebraic summation of the two potentials (fig. 9). Eccles has shown that the motor neurone is absolutely refractory for 2.5σ and relatively so for 10.5σ, therefore if any of the waves in question were in the motor cells, their size should have been decreased. Using the same method, Gasser and Graham showed that motor cells showed no potential resembling the smooth form of the negative portion of the cord potential. The present experiments now exclude them as the source of the shorter lasting potentials which at times modify the smooth form.

The second negative complex. A second wave recording in the negative direction starts about 25σ after the beginning of the response (i.e., at the time of the crest of the positive wave) and lasts until 50σ. It is not peculiar to any special state of the cord but deep narcosis prevents its appearance. In light narcosis and in the less irritable decerebrate and spinal preparations it appears with a smooth contour tending to obscure the positive wave otherwise present. Like the first negative wave, the second can develop shorter waves on its surface. The best instances have been seen in decerebrate preparations, one of which is reproduced in figure 4*a*.

SUMMARY

The variations in the potentials occurring in the internuncial neurones of the spinal cord, after application of a single induction shock to a dorsal root, e.g. L_7, are described.

Cords with low irritability show a basic potential-form made up of a negative wave followed by a longer positive wave. Cords with high irritability develop a potential in which there are added to the basic form numerous shorter waves recording mostly in the negative direction. The latter cannot be in motor neurones as they are unaffected by antidromic impulses from the sciatic nerve.

The negative potentials can be divided into two complexes, of which the first, which lasts for about 25σ, is associated with a discharge of impulses into the sciatic nerve. Activity of the motor neurones can be seen only

when the cord potential is relatively simple in form and then appears as thin crescentic indentations in the first complex; otherwise it is completely obscured by the intermediary potentials.

The positive wave, like the negative wave, is generated after the point at which the pathways from two roots overlap. The neurones in which it occurs are mainly in connection with low threshold fibers in the roots.

Paired leads from different positions on the cord yield potential-forms compatible with the assumption that the positivity is due to a changing position of the locus of maximum negativity in the generating units. Other interpretations are not excluded.

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THE RESPONSE OF THE SPINAL CORD TO TWO AFFERENT VOLLEYS

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Under controlled experimental conditions the potentials initiated in the spinal cord by an afferent volley are sufficiently reproducible, as to form and magnitude, to permit studies of their variations under changing conditions. One of the most instructive variations is that occasioned by existing activity. If a volley be so placed that it arrives at the first synapse at various times after a conditioning volley, the resulting potential is seen to have a form dependent upon the interval and the nature of the residual potentials in the cord. By comparing the recovery curve with that of peripheral nerve it is possible to obtain an idea of how far the internuncial neurones behave like a typical irritable tissue subject to periods of absolute and relative refractoriness; and, by comparing the course of the cord potentials with the size of the homolateral flexor reflex elicited by the paired stimuli, some hint may be gained as to the rôle of the internuncial neurones in the reflex mechanism.

METHODS. In the experiments recorded in this paper all the leads were made from the dorsum of the cord. The electrical technique was the same as that described in the preceding paper except that provision was made for the application of two induction shocks in succession. Both were thyratron-controlled, according to the method of Schmitt and Schmitt (1932), and were usually applied to a single root, as L_7. In some experiments two adjacent roots were employed. Because of the tendency of the cord to undergo spontaneous changes in irritability during the course of a series of observations, it was found necessary to take three records at each interval of stimulation: one of the isolated conditioning response, one of the isolated testing response, and one of the two in succession. The three films so obtained could then be superimposed for examination of the changes produced.

Many of the changes are susceptible of only descriptive treatment, but in the case of the first negative wave curves are plotted. The method of measuring is as follows. The heights of the isolated negative waves are taken from a projection of the base line and are marked, respectively,

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C (conditioning) and *T* (testing) in the figures. For the conditioned response the resting base line is not a suitable level of reference as the information desired is the addition of potential occasioned by the testing shock. To obtain this the negative crest must be measured from the level the isolated conditioning response would have at the time in question, whether the latter be above or below the resting level.

When the flexor reflex was recorded in addition to the electrical picture the tibialis anticus, on the same side as the root stimulated, was isolated and attached to a tension lever after the necessary denervation of the other muscles of the leg and fixation of the tibia with drills held in clamps. The lever used was of the torsion-spring type, writing on a smoked drum. It had an undamped frequency of 60 and therefore was able to measure the tensions with as great a degree of accuracy as was necessary for any of the conclusions which were drawn from the measurements. The total tensions of the contractions produced by the double stimulations were plotted for comparison with the tensions of the isolated reflexes.

RESULTS. Before attempting an analysis of the experiments a brief survey of their contents will be given. In this survey, wherever possible, descriptions will be confined to the observations made in a single experiment (designated for reference as experiment I) so that the pointing out of correlations may be facilitated; but the features described are all confirmed by other experiments.

Following the activity evoked by an afferent volley two alterations in the response of the cord neurones appear, which have counterparts in the behavior of peripheral nerve. The first is a period of unresponsiveness. To show the latter, records selected from one series of observations made during experiment I are arranged in figure 1. Record 1*a* shows the

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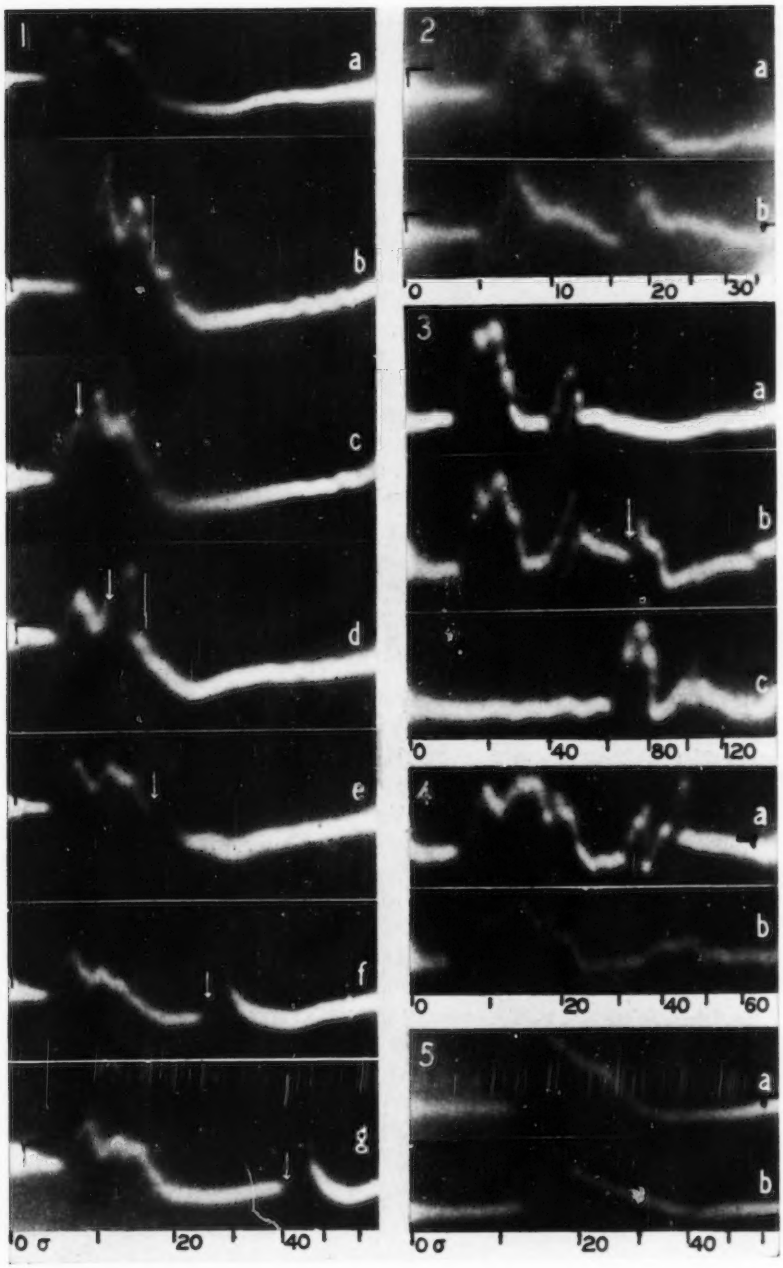
Fig. 1. Effect of a weak conditioning stimulus, *a*, on a strong testing stimulus, *b*, at intervals of 4σ, *c*; 5.5σ, *d*; 12.5σ, *e*; 21.5σ, *f*; 37.5σ, *g*. Position of testing shock marked by arrows. Spinal; no ether for one hour (4/18/33).

Fig. 2. The effect of two volleys at an interval of 12.5σ on the same preparation in two experimental states. Same preparation as in figure 1. *a*, spinal. *b*, intact central nervous system, light ether anesthesia.

Figs. 3 and 4. Cord potentials produced in an active decerebrate preparation by two afferent volleys of equal strength. Ether administration had been discontinued 2 hours previously. 3*a*, response to conditioning shock. 3*c*, response to isolated testing shock. It is like 3*a* but on a different time scale and occupies the position which the response to the testing stock holds in 3*b*. 3*b*, response to the two shocks at an interval of 61σ. 4*a*, response to isolated testing shock. 4*b*, response to the testing shock following a conditioning shock by 880σ. The response to the conditioning shock would be off the screen to the left and have the same form as 4*a*.

Fig. 5. Prolongation of the negative component of the cord potential occasioned by previous activity. *a*, response to isolated testing shock. *b*, response to testing shock following conditioning shock by 130σ. The response to the conditioning shock is off the screen to the left. Intact nervous system; light ether anesthesia (9/12/33).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Figs. 1 to 5

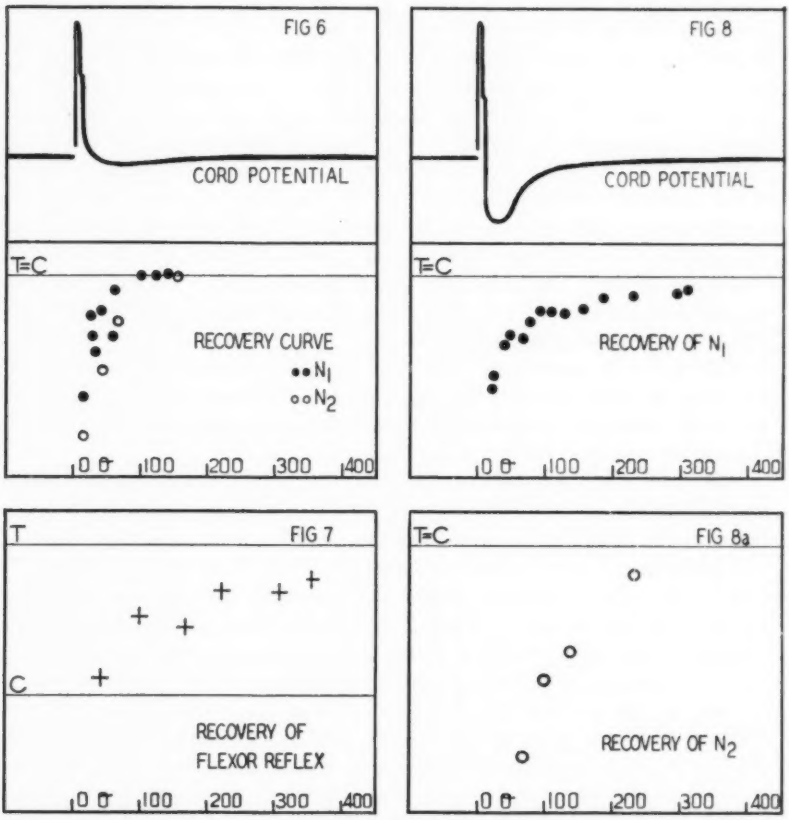
response to a weak conditioning shock; 1*b*, that to a stronger testing shock. It will be noted that the negative component of the latter is considerably larger but that there is a very small increase of the positive swing. In the succeeding records are seen the responses when the two shocks are applied at various intervals. All of them show that the potential added by the testing shock is less than that contained in the control (1*b*); but the full deficit does not develop immediately. When *T* falls at the first negative crest of *C* (at the arrow in record 1*c*) the decrease in size of its negative crests is small. When it falls at the start of the second negative crest of *C* (record 1*d*) the decrease is larger but the later crests are still visible. Longer intervals increase the deficit and by the end of the series the later crests have quite disappeared.

The second similarity to peripheral nerve is illustrated in figure 5. Part 5*b*, which in isolation should look like 5*a*, follows a conditioning response by 130σ and shows a prolonged negative complex encroaching on what would otherwise be the early part of the positive wave. In nerve, a conducted wave is prolonged by the increased temporal dispersion resulting from the subnormal velocities holding during the relatively refractory period and the dispersion seems to be imitated in the cord. As a result the constituent waves become separated. In figure 5*b* the first crest is made more prominent by the delay of the second wave; in the records of other preparations component crests appear which are not visible in unconditioned responses and which can be proven not due to fortuitous variations.

The amount of deficit in a conditioned response depends upon the state of the cord. Two shocks placed at identical intervals may produce different effects in a single preparation if the experimental state of the animal be altered in the intervening period. In figure 2 there are placed for comparison two more records from experiment I: one (fig. 2*a*) made 10 minutes before the series used in figure 1, that is, after the preparation had been made spinal; the other (fig. 2*b*) made during an earlier period in which the central nervous system was still intact but in which there was a light ether narcosis. In both there is a large deficit in the testing response (visible when compared with the isolated testing responses which are not shown); but in the former the later negative components are obliterated while in the latter they are only reduced.

Recovery of responsiveness occurs according to characteristic curves; but the form of the curves, like the degree of maximum deficit, varies with the condition of the cord. In general, cords giving the least elaborate responses to stimulation recover more rapidly. When the positive wave is undeveloped, as in figure 6, recovery of the negative wave is so rapid that no detectable deficit remains at 100σ; on the other hand, when the positive wave is well developed (fig. 8) recovery, though initially resem-

bling the former case, later slows down and a deficit is still measurable at 300σ. In still more irritable cords, in which there is not only a multiple-crested first complex but also a striking second complex, recovery even of the early waves is still slower and all the components are involved. The reactions of an irritable cord at two separations of the stimulating



Figs. 6 to 8

shocks are illustrated in figures 3 and 4. At an interval of 61σ (fig. 3) there is a big decrease in the first complex and an almost complete obliteration of the second. At 880σ (fig. 4) the subnormal form of the first complex is still definite (proven by records in triplicate) and the deficit of the second complex is gross.

Further details concerning the recovery of the cord potentials after activity appear in figure 9, which is arranged also to show the correlation

of the potentials and the flexor reflex. The data are obtained from two series of observations made in experiment I: the first, under light ether anesthesia; the second, after the preparation had been made spinal. At the top of each part of the figure is an electrical record of the conditioning activity. In both cases the testing response represents the effect of a shock stronger than the one used for conditioning. When in isolation the form holding for the spinal animal is as in figure 1*b*. Below the action potentials are plotted the heights of the first negative crests (N_1), measured as described in the paragraph on methods. The heights of the second negative crests (N_2) cannot be measured, as N_2 cannot be differentiated from the potential belonging to N_1; but the absolute height is plotted in the figure as open circles in order to give a rough idea of the activity of the neurones responsible for it. In the first series recovery of N_2 starts approximately with N_1, and at 30σ is about 70 per cent completed. In the second series recovery does not start until after 50σ, the last point at 74σ being the only one indicating any recovery at all. To show how this potential would recover in such a preparation a curve taken from another animal is shown separately in figure 8a. In the latter N_2 starts to recover at 650, which is much later than the beginning of recovery of N_1 in the same preparation (fig. 8). Throughout the earlier portion of the recovery period the recovery of N_2 is less advanced than that of N_1; but final recovery apparently comes to them both at the same time.

The records of the spinal cat are characterized by a good positive wave and the increments added by *T* to *C* can easily be measured, therefore a recovery curve of the positive wave is included in this part of figure 9. The positive waves on the control curves, *C* and *T*, are much alike, therefore there is little positive potential going with the negative potential elicited in *T* but not in *C*. When *T* follows *C* the negative potential added from neurones not involved in *C* would for this reason not be expected to cause an addition of positivity and none appears; the added negativity is written on the positive wave of *C* as a base line. Only when the conditioned neurones begin to recover and negativity begins to appear above the line $T-C$ do measurable increments of positivity occur. Both potentials continue to improve up to the time that all electrical activity produced in the cord by *C* has subsided and with considerable similarity of their recovery curves (compare the curve of positive potential in fig. 9, series 2, with the part of the curve of negative potential above the line $T-C$). This similarity strongly suggests that the positive and negative potentials arise in the same neurones and that both potentials are deficient because neurones are failing to respond or are responding subnormally.

The lower sections of figure 9, in which the size of the flexor reflex is recorded, show very different curves. In the first series over a period of 30σ the reflex is larger than the sum of the two controls; in the second

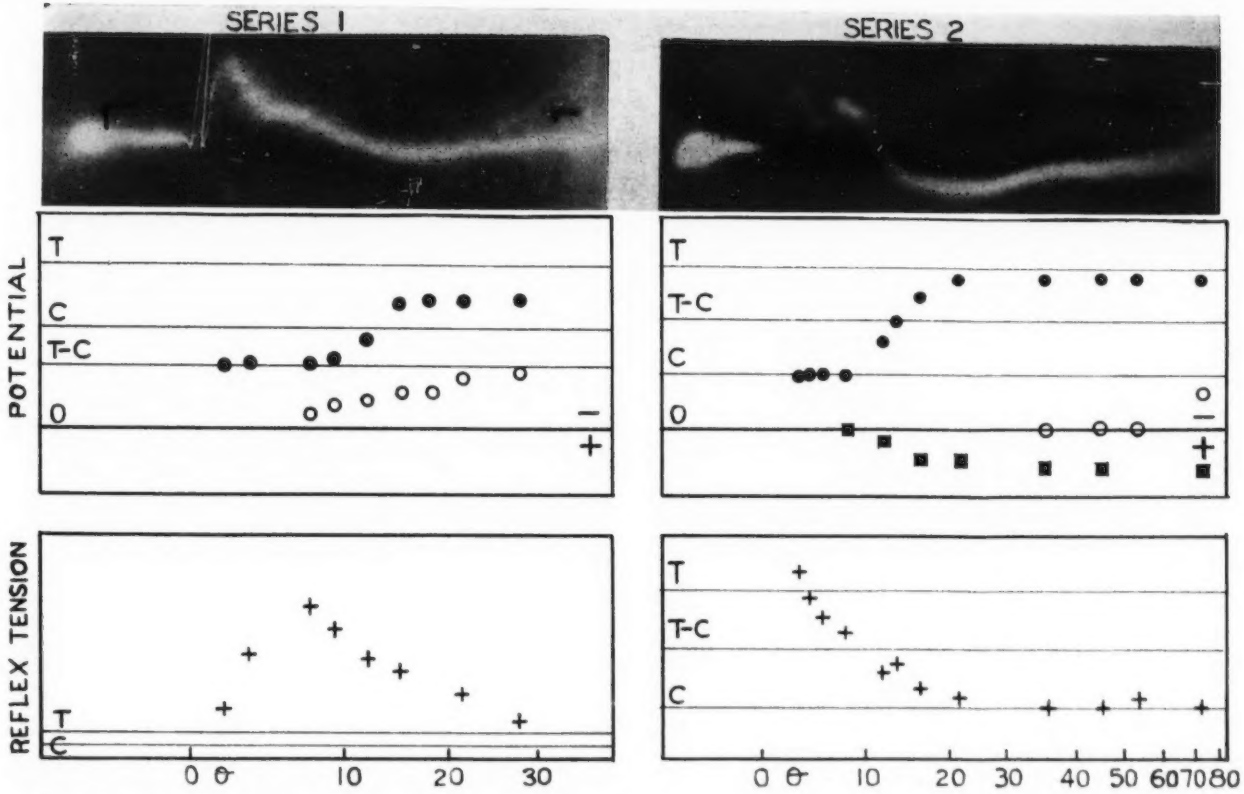


Fig. 9. Comparsion of cord potentials with the tension developed in the tibialis anticus muscle during a homolateral flexor reflex. Records, responses to conditioning volley. Potential graph, magnitudes of waves in testing response: ●●●●, N_1; 0000, N_2; ■■■■, positive waves. Ordinates arbitrary. Series 1, intact nervous system; light ether narcosis. Series 2, spinal (4 18 33). Other explanations in text.

every point after the first falls below the tension of the testing control. At about 20σ T ceases to add anything to the total tension and recovery has not started at the end of the curve. In order to supplement the latter a recovery curve from another experiment is given in figure 7. In this experiment a rapid drum was used so that the actual tension attributable to the testing shock could be measured by the method used by Eccles and Sherrington (1931) and attributed to Eccles and Hoff. Numerous observations were made at each interval so as to obtain valid readings for the late points and they show that recovery was not yet complete at 350σ. The positive wave on C lasted to this time but was becoming indeterminate because of its asymptotic ending, and the negative waves on T were approaching but had not quite reached recovery.

The two series shown in figure 9 are typical of two types of reaction and in addition show a transition from one type to the other in a single animal. The two types are characterized by the potential curves. A relatively simple negative wave, followed by an undeveloped positive wave, goes with reflex summation; and a negative wave divided up into component crests and followed by a well-developed positive wave goes with reflex depression. In the experiments cited the ratio of C to T is not the same, the stimulation strengths being selected to bring out the differences between the two series. The difference itself, however, is only in part due to the sizes of the control responses, for it is impossible by any combination of strengths of stimuli to imitate the myograph curve of the first series when the second type potential obtains, and vice versa. While the cord potentials must represent much activity not involved in the flexor reflex, still they present backgrounds so typical that from the potential pictures alone it is possible to predict almost without error what the myograph will show.

Analysis of experiments. The first step to be taken toward an understanding of the recovery of the structures in the cord is a consideration of the excitation these structures receive. The stimulation of the first neurone is controllable and follows the laws for peripheral nerve. From the latter the important points to bear in mind are the smaller sizes of the responses during the relatively refractory period and the greater-than-normal magnitude of the shocks necessary to obtain them. It has been pointed out repeatedly (Sherrington and Sowton; Adrian and Olmsted; Forbes) that no further activity in the spinal cord is to be expected until the nerve recovers. The nerve, however, is not the controlling factor in the experiments here discussed as the intervals are long enough for the spikes of the second volley to be full sized. Beyond the first neurone there must be a more slowly recovering section of the pathway. To measure properly the recovery curve of a tissue in such a position is impossible as the measurement would involve control of the strength of

stimulation. Secondary neurones cannot be reached directly, and their connections with primary neurones must be very variable. Neurones in a direct path would receive many terminations, while those in more indirect paths would receive terminations correspondingly fewer in number; and excitation normally would range all the way from supramaximal to subthreshold (Sherrington, 1929). When the neurones are once brought into activity those in the indirect lines could therefore be re-excited by an oncoming volley only after a longer interval than would hold for the neurones in the direct lines, for the same reason that a peripheral nerve fiber takes longer to recover to the point of response to a weak shock than it does to a strong one. Among the cord potentials the ones slowest to recover are those forming the later negative components: that is, those normally developing after the longest latency and most easily lost in states of narcosis—and for these reasons to be considered as having the smallest number of excitatory *boutons* on their surfaces. Their late reappearance may as well be due to the necessity of a higher degree of recovery as a slower rate of recovery.

A cord neurone, like any irritable cell, should give evidence of a refractory period; but tests of such refractoriness are complicated by the fact that they must be performed in the presence of other neurones which have received a subthreshold amount of excitation and are therefore subject to facilitation. In peripheral nerve the period of facilitation is too short for any axons not excited by a submaximal conditioning shock to add their potentials to recovered axons stimulated by a testing shock; but abundant myographic evidence (Forbes, Querido, Whitaker, and Hurxthal; Eccles and Sherrington; Bremer) shows that one cannot count on such a fortunate experimental situation in the spinal cord. Furthermore, there is the complication designated as inhibition. In the face of any precise definition of the latter it is necessary to be arbitrary, and we have set our criterion of inhibition to be a response-deficit not directly attributable to refractoriness.

While it is impossible to evaluate the rôle that each of the possibilities—facilitation, refractoriness, and inhibition—play in any experiment, it is possible to set a limit to the extent to which any one of the qualities may be invoked in an explanation, especially when the conditioning shock is the weaker one. When the C neurones are completely refractory one should still be able to excite the T neurones not included with C and a potential $T-C$ should be obtained. Thus points falling at $T-C$ would be adequately explained by absolute refractoriness of C neurones, and points above it by their partial recovery; but points falling below this line must mean that neurones not previously active have failed to respond. With this formulation in mind it is apparent that the unresponsiveness of the internuncial neurones seen in series 1 of figure 9 can be explained as refrac-

toriness, but that, for the points falling below $T-C$ in series 2, something more than simple refractoriness must be invoked. By definition this something would be inhibition.

Theoretically points might occur above the $T-C$ line because of facilitation or of partial recovery of C neurones. If due to facilitation one would expect points to appear in the first 10σ, both on account of the fundamental mechanism of facilitation and the occurrence of facilitation of the flexor reflex in this period (Eccles and Sherrington, 1930, 1931). No points appear in this region, however, even in experiments in which facilitation of the flexor reflex is proven; therefore, when points do begin to appear above the line, they probably mean recovery of C neurones. There is evidence of facilitation of the negative waves in the second complex but it has not been analyzed.

In the interpretation of the reflex experiments the tensions reached may be taken to indicate the number of motor neurones activated. If the number of nerve fibers involved in T were stimulated at the crest of the C twitch, according to the observations of Cooper and Eccles, the tension would be $T+C$. Reflex tensions greater than $T+C$ would therefore mean additional active axons in the testing volley or facilitation. This criterion of facilitation permits no false inclusions, but it is more than necessarily rigid for greater separations of the constituent volleys as, instead of C, which is the maximum tension, the momentary tension would be sufficient. As the records, except for the instance noted, were in straight lines, the more rigid criterion is the only one possible; but the number of border-line cases excluded is not large because of the small size of the conditioning shock, so small in some experiments as by itself to produce no reflex at all. Reflex tensions somewhat less than T could mean refractoriness of neurones involved by the conditioning volley; but if they fall below $T-C$ inhibition must be postulated. Here again the criterion covers the limiting case and is too rigid for the others.

As others have found, the effect of the conditioning shock may be a pure facilitation of the flexor reflex, a facilitation followed by a depression or a pure depression. Application of the foregoing criteria to series 1 of figure 9 shows that the reflex is marked by facilitation. Facilitation starts as soon as the second shock falls outside the refractory phase of the root, but does not reach a maximum until the region of the second convexity on the negative part of the cord potential. From then on it progressively declines. Application of the same criteria to series 2 shows that inhibition is recognizable after 10σ and continues to the end of the experiment.

Refractory period of the reflex arc. Further analysis demands a consideration of the duration of the refractory period of the neurones in the reflex arc and the extent to which deficits of potential and tension may be explained by it. In the frog Bremer has derived from the curves of reflex

summation a probable value for the refractory period of the internuncial neurones; but in the mammal the only part of the reflex arc about which there is any definite knowledge is the first and last neurone. The refractory period of the axon of the first neurone is well known; the cell body is not involved and the endings must be like the axon, otherwise the refractory period of the axon would not be a limiting factor in reflex summation. The last neurone has been examined by Eccles. With the aid of antidromic impulses he has obtained data indicating that the neurone is absolutely refractory for a period not longer than 2.5σ and relatively refractory for not longer than 10.5σ. In comparison, the internuncial neurones appear to recover more slowly. Figure 9 fails to show any negative potential which may be attributed to recovered neurones until 10σ have elapsed. How close this duration comes to the absolutely refractory period depends entirely on how early in the recovery period such stimulation as one is able to give these neurones is able to elicit a response.

Recovery once started, the potential mounts rapidly to the region of 100σ, after which it gains slowly for a variable period. Whether the deficit in the second response comes within the possible limits of refractoriness or necessitates the assumption of inhibition as well, the recovery curves of the first negative crest are very much alike. In fact, it would be necessary only to equalize the two volleys in an experiment showing "inhibition" to eliminate any deficit which could be interpreted as such under our working definition. Furthermore, there is no point on an individual curve which marks a separation between refractoriness and inhibition. In other words, there is no evidence other than the end result that the unresponsiveness is accomplished by more than one process.

The question at once arises as to how far refractoriness alone could explain the phenomenon attributed to "inhibition." If all the unresponsiveness be ascribed to the refractory state, then neurones, once activated, would not recover until the end of their relatively refractory periods, which might last 400σ; and those previously unexcited would have to be considered as being prevented from responding to a testing shock (inhibition) because of refractoriness of internuncial neurones strategically placed in the pathway in some such fashion as shown in the accompanying diagram. Let *a* (fig. 10) represent low threshold axons stimulated by the conditioning shock and *b* higher threshold axons reached by the stronger testing shock; and suppose two endings must be active for excitation to take place. *a* will stimulate neurone *1*; $a+b$ will stimulate *1*, *2*, and *3*. Neurone *2* is included by summation. If now *a* precedes $a+b$, neurone *1* will be refractory and $a+b$ will fail to stimulate *2* on account of the missing ending. This would in turn result in inhibition of the flexor reflex because of loss of excitation to a motor neurone by neurone *2*. The foregoing hypothesis fits particularly well the later components of the first negative

complex as their other qualities indicate them to be in more indirect connection with the sensory root.

Intermediary potentials and facilitation and inhibition of the flexor reflex. Absence of any signs of facilitation of the negative intermediary potentials in the presence of marked facilitation of the flexor reflex indicates very strongly that the latter occurs in the motor neurone. In this respect the indication supports the localization ascribed to the phenomenon by Eccles and Sherrington (1931) as the result of their observation that "c.e.s." is inactivated by antidromic volleys. If we think of the motor neurones as being excited through internuncial neurones, then we have to account for the facilitation in the face of a deficit of intermediary potential belonging to the testing shock. This necessity presents no great difficulty, however. The potential, although reduced in size, when added to the potential belonging to the conditioning shock produces one with an area

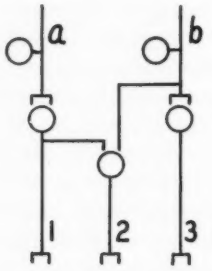


Fig. 10

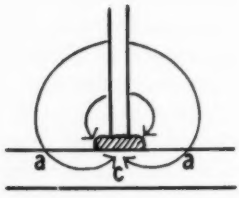


Fig. 11

greater than either constituent and with a longer duration. Thus, just as a long condenser discharge has greater stimulating power than a shorter one of the same potential (and some with a higher potential), a larger number of motor neurones should be caused to discharge. (The stimulation at the surface of the motor neurone is more probably by a fusion of multiple discrete and asynchronous impulses, but that fact does not destroy the force of the analogy.)

The period of facilitation extends beyond the point at which conditioned neurones begin to recover but with a declining intensity, in spite of the fact that the area of the negative potential is increasing. This relationship is also reconcilable with the general laws of excitation. A subthreshold applied potential causes an increase of irritability, which rises to a maximum and then decreases, so that the size of an added stimulus necessary to bring excitation to threshold after reaching a minimum begins to increase and, if the conditions are correct, finally becomes larger than normal (Gildemeister; Bishop; Erlanger and Blair). If the motor neurones act

like other irritable tissues it is quite understandable that the increment of stimulation from the testing volley should be less effective as the impulses arrive later and later. At a sufficient interval the effect of the first stimulus should die out and the final response become more dependent on the quality of the second stimulus. This fact furnishes the clue for the transition from facilitation to inhibition.

In order to understand the conditions which must obtain for inhibition to be manifest it is necessary to examine in more detail the differences between the two series in figure 9. In the first series, showing facilitation, the later components of the first negative complex are poorly developed; but, compared with the second series, they are less readily affected by a preceding volley, so it is much easier to add potential area from the two volleys (fig. 2*b*). In the second series, giving inhibition, the later components are well developed but they are readily suppressed by a preceding volley even when the latter is much smaller (fig. 1*f*). Under the conditions of the experiment the afferent testing volley is made large so as to evoke a large intermediary potential and a large reflex, while on the other hand the conditioning volley is small. In the combination, therefore, the important portion belongs to the testing volley. We may now consider the situation holding when *T* falls 40σ after *C*. The intermediary potential, divested of all the later components, impinges on the motor neurones too late to sum with the excitation from *C*, and has only such stimulating power as it itself possesses. This is very little, as the reflex is no larger than the *C* volley alone could produce. Further proof of involvement of the later components in production of reflexes is found in the early part of the curve of reflex tension (series 2, fig. 9). The curve starts out just above the *T* level but progressively declines to a minimum which is reached soon after 20σ. The decline fits very well with the mode of elimination of *T* potential by previous activity, for we have seen that the deficit increases as *T* is placed later in the *C* potential and that the later components are not erased until the end of the negative part of the latter. All during the summation period, therefore, the later components are able to contribute something to the excitation of motor neurones.

A comparison of the reflex curves in figure 9 with the potential curves shows that the contrasting forms of the reflex curves have no counterpart in the recovery curves of the first negative crests but do, on the contrary, accompany a marked difference in the time of restoration of the later crests. In the preceding paragraph it was shown that full reflex inhibition does not develop until the stimulus interval is sufficient for maximum elimination of the later negative components of *T*. It is also true that release from inhibition does not begin until the latter start to recover; then restoration proceeds along a curve much like that shown for N_2 in figure 8*a*. These considerations indicate that "inhibition" of the flexor

reflex, as it is seen in the present experiments, is largely due to failure of excitation to reach the motor neurones. Incidentally the experiments afford the best electrical proof available of the involvement of internuncial neurones in reflex activity.

The foregoing simple formulation cannot be taken as a complete explanation of inhibition, however, even within the limits of the data presented in this paper, for no weight is given to the persistently occurring correlation between the mode of recovery of the intermediary potentials and the magnitude and duration of the positive wave. Now whatever interpretation one may put upon the nature of the wave, none of them can negate the conservative conclusion that a potential picture cannot be reproduced in the cord until all trace of the preceding picture has died out. This fact in itself gives a hint as to the possible nature of inhibition. Theories concerning the nature of inhibition have been historically of two types: those making use only of the properties of peripheral nerve—notably those of Lucas and of Forbes—and those postulating chemical intervention. In behalf of the first type of theory the significance of the long duration of the cord potentials must be apparent, as the new set of constants obviates the necessity of making the complicated assumptions which have to be made to build a theory from the constants of peripheral nerve-spikes. Chemical intervention is not suggested by any of the observations made in this investigation.

Association of irritability changes with potentials of long duration leads to a consideration of the effects that might occur by polarization, since the latter can theoretically be produced by the local bioelectric currents arising from any structure developing a potential. Polarization of the proper strength and duration produces excitation; otherwise depression occurs. In the absence of any known difference in the qualities of nerve terminals other than their position on the surface of the neurone the only variable with which we have to work is the strength and duration of their activity—but that is sufficient. The persistent negativity of a *bouton* ending on a dendrite may be considered in connection with figure 11. Local bioelectric currents between it and the parent fiber cross the plasma membrane at *a* in such a way as to produce anodal polarization and therefore depression. Masses of such terminations in activity could effect stimulation through summation of cathodal polarization at *c*. Failing excitation, the increased irritability produced in the cathodally polarized region would progressively decrease again (Bishop; Erlanger and Blair), and outside of it anodal polarization would persist during the duration of the potential. The threshold to subsequent volleys would be raised and *boutons* strategically placed on a dendrite might block an impulse arising from stimulation of its terminals. Indeed, when we consider the wealth

of interlacing in the neuropile, it is conceivable that depression by polarization may be widespread.

SUMMARY

As measured by excitation through the first neurones, the absolutely refractory period of the internuncial neurones in the lumbar enlargement of the spinal cord of the cat lasts 10σ.

Relative refractoriness, together with any other possible factors making for unresponsiveness, lasts a minimum of 100σ; and durations much greater are common (one second and longer).

An afferent volley may render neurones unresponsive to a subsequent testing volley without causing them to become active. This may be explained by the interposition of refractory neurones in the pathway of the impulse.

The background out of which a flexor reflex arises is sufficiently distinguished by the cord potential to make it possible to predict from the form of the latter whether a reflex produced by a single afferent volley will be facilitated or inhibited by an antecedent volley.

Inhibition of the flexor reflex under such conditions can, for the most part if not entirely, be explained by blockade of internuncial neurones necessary for transmission of excitation from the primary to the motor neurones.

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SUPERFETATION IN THE ALBINO RAT[1]

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It has been previously shown (Wang, 1923; Slonaker, 1924) that the normal female rat during sexual life shows rhythmic fluctuations in spontaneous activity which correspond with that of the reproductive rhythm. It was further demonstrated, both by vaginal smears and mating tests, that these spurts of activity were synchronous with estruation and could be relied upon as indicating the occurrence of this reproductive phenomenon. It has been shown also (Slonaker, 1924) that generally during the period of normal gestation these rhythmic fluctuations in activity do not occur and the spontaneous activity is reduced to a low amount. However, in the study of many individual curves of activity during gestation an occasional spurt of activity, characteristic of estruation, was found to have occurred usually between the twelfth and fifteenth day after coitus. No vaginal smears nor mating tests had been made, but, owing to the establishment of the synchronous relation of these activity peaks with estruation, we at that time concluded that ovulation had taken place. Since then we have been looking for verification of that assumption.

Two cases have been noted, which, in our opinion, can be explained only by assuming that ovulation and fertilization had occurred during the period of pregnancy. The histories of these cases follow.

Case 1. A male and female rat were placed in a stationary cage. The male was removed from the cage twenty-one days later. The following day the female gave birth to five normal young, all of which she ate within three days after delivery. Isolation of the female was continued and fifteen days after the above delivery a second litter of six normal young was born. These the mother reared. Since the period of gestation in the rat is approximately twenty-two days the ovulation and fertilization which resulted in the second litter must have taken place some fifteen days after that for the first litter.

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Case 2. A male and female were mated at the ages of 44 and 23 days respectively. When the female had attained the age of 111 days she delivered a litter of four males and five females. These young were found at 11 a.m. and had nursed. This indicated that they had been born some hours previously. Three of these young died within three days. The remaining six were reared. The male of the pair was removed from the cage when this litter was discovered. The female and her litter were thus left isolated during the nursing period. Fourteen days after the birth of the first litter at 5 p.m. a second litter was being delivered. This consisted of three normal young, two males and one female. This second litter was crowded out of the nest by the first litter and did not have a chance to nurse. One male and one female were eaten by the mother and the other male died and was removed from the cage. The remaining six of the first litter made a normal growth and were weaned at the age of twenty-five days. The only explanation which seems reasonable that will account for these results is that an ovulation, fertilization and implantation occurred on approximately the fifteenth day of gestation.

These are the only authentic cases we have observed that justify our former assumption that the estrual-like spurts in spontaneous activity of rats, which occurred occasionally during gestation, indicated true ovulation. Other instances may have happened which escaped our notice due to the young having been born and eaten by the mother during the nighttime. These two cases seem to prove conclusively that superfetation does occur in the rat.

We can only conjecture as to the arrangement of the young of these two litters in the uteri. Either the first litter was confined in one uterus thus leaving the other uterus free for the implantation of the second litter, or the young of the first litter were situated in both uteri and the sperm of the second coitus were able to pass the developing young of the first litter and fertilize the ova beyond. In the latter case the young of the second litter would be implanted nearer the oviduct. In either case delivery of the first litter could take place without serious injury to the second immature litter. It is hoped that further investigations may be able to give some information in regard to this.

SUMMARY

Two cases of superfetation in the rat are given which substantiate our former assumption that the estrual-like peaks in spontaneous activity occasionally found during the gestation period represent true estrus.

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AGE VARIATIONS IN THE POLYMORPHONUCLEAR LEUCOCYTE OF THE RAT[1]

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Classification of the human segmented neutrophil cells according to their age, as indicated by the number of nuclear lobes, was early advocated by Arneth (1904). Later Schilling (1929) presented a count, using a classification of the earlier unsegmented granular cells. Of late years the latter method has been considered somewhat more reliable and sensitive as an indicator of infection and body resistance thereto. Recent comparative studies conducted in this laboratory on Arneth and Schilling counts made simultaneously on normal human subjects over a period of months, directed our interest to a similar application in an experimental animal for both expediency and better controlled experiments.

Application of the Arneth count to the cells of the rat presents many possible variations owing to the extreme diversity of the rat neutrophil nuclei. After considering those forms found in the blood of weanling rats, together with smears from the bone marrow and the blood of adult animals, we have divided the neutrophils into five groups. These groups number as in the Arneth human counts, but are based on somewhat different criteria. Owing to the skein-like nature of the rat neutrophil nucleus the lobulations are not constantly present and are frequently unintelligible. For example, a cell of maturity in adverse circumstances, as evidenced by early pyknosis, may present a knotted nuclear skein of grossly two lobulations, but in reality it will be numerous twists contracted into the bi-lobed appearance. In order to eliminate this evident discrepancy in Arneth counts on the rat, we have assumed the following arbitrary criteria, based primarily on the degree of looping rather than the gross lobulations. The following applies only to the neutrophils.

Group I. All globular or fully rounded nuclei are included herein. No attempt is made to subdivide these early unsegmented cells, as in the Schilling count. The shape of the nucleus is considered rather than the graded staining reaction. These young cells usually, but not always, contain granules that tend to be more acidophilic than those of the older

[1] The authors acknowledge the valued assistance of Miss Grace Heimann in this work.

neutrophil cells. The granules are somewhat larger in these early cells. These cells are common in bone marrow preparations (plate I, figs. 1 and 2).

Group II. The circinate or doughnut shaped nucleus is readily identified. This nuclear form is commonly found in the adult eosinophil of the rat. There may be notching of the circular structure, but so long as

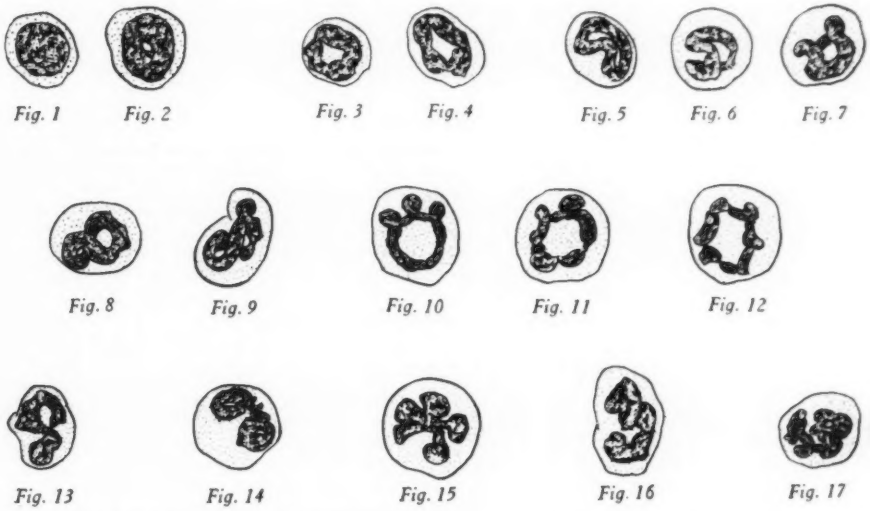

Plate I. Camera lucida drawings of the various types of neutrophil nuclei found in the rat.

Figs. 1 and 2. Early globular forms. Group I.

Figs. 3 and 4. Circinate or doughnut shaped nuclei. Group II.

Figs. 5, 6 and 7. Forms of early nuclear looping. Note the loops approximate, but do not touch. Group III.

Figs. 8, 9, 10 and 11. Figures-of-eight and early looped forms. Note the loopings of the circular form are not sharply drawn, nor exceed five in number. Group IV.

Figs. 12, 13, 14, 15, 16 and 17. Various types of group V loopings showing the tangled skein appearance of the mature nuclei. The looped circular forms classed under this group have loops in excess of six and are more tightly drawn and tend to become angular.

there are no gross variations the cell is considered as of this group (plate I, figs. 3 and 4).

Group III. A partial twist, or incomplete looping of the nucleus of the previous type constitutes group III; that is, an early figure-of-eight form with the loops at right angles to one another in either plane, or a circinate form folded on itself, but without the loops touching, rather approximating (plate I, figs. 5, 6 and 7).

Group IV. This group includes the fully formed loops-on-themselves and true figures-of-eight nuclei, together with the circular forms which have become irregular from looping, but still may be classed as a ring. The circular forms of this group are to be differentiated from those of the succeeding group in that the loops are five or less in number and are not sharply angled or tightly drawn as they are in group V (compare figs. 11 and 12) (plate I, figs. 8, 9, 10 and 11).

Group V. Any further looping, knotting, or bulging of the simple nuclei described as of group IV are placed in group V. Looped circular forms with angular or tightly drawn loops are included in this group.

TABLE 1

Average blood findings with the age variations of the Arneth count in the rat*

AGE, DAYS	HEMOGLOBIN, GRAMS PER 100 CC.	TOTAL WHITE BLOOD CELLS	ARNETH INDEX	PER CENT								
				Neutrophils					Total neutrophils	Total lymphocytes	Monocytes	Transitional and unclassified
				I	II	III	IV	V				
21	12.3	6565	32.34	14.08	12.60	12.40	18.30	42.40	10.7	80.5	4.8	2.5
28	15.1	7676	18.94	5.43	6.17	14.69	26.30	46.80	15.8	75.5	5.2	2.4
35	14.8	7755	21.10	4.34	7.43	18.65	23.63	45.60	7.3	85.5	2.6	2.7
42	15.0	7020	18.46	1.67	7.66	18.26	26.80	44.70	9.3	86.8	2.6	1.2
49	15.3	12543	14.29	0.98	5.06	16.49	25.41	51.98	12.0	80.8	3.5	2.8
56	16.7	12700	14.23	1.76	4.77	15.40	26.56	51.50	11.8	80.1	4.4	3.2
63	15.0	10572	11.04	0.32	4.84	11.77	22.26	60.74	14.8	80.3	3.9	3.1
70	15.2	9880	8.23		1.91	12.63	28.15	57.20	18.2	74.0	3.7	2.4
77	15.3	9368	8.87		1.20	15.35	30.06	52.70	12.3	77.9	4.4	3.1
84	15.6	11350	7.77		1.17	17.21	25.34	56.26	15.6	78.3	3.8	1.3
91	14.8	8940	6.91		1.57	10.68	25.10	61.98	18.0	75.0	2.9	2.3
98	15.0	10022	7.82		1.66	12.33	26.60	59.40	16.9	77.3	2.4	1.3
Av....	15.0+	9533	14.17	2.38	4.67	14.65	25.38	52.60	13.5	79.3	3.7	2.4

* Five to ten determinations.

The number of loops are more numerous (six or more) than are the circular forms described under group IV above (plate I, figs. 12, 13, 14, 15, 16 and 17).

Further classification of the cells on the above nuclear basis becomes too complex, inaccurate, and unreliable. In considering cells for the above groups, any cell of questionable classification is placed in the next succeeding group.

In order that a numerical evaluation of the count might be given for graphical purposes, Arneth offered the summation of the first two groups as an index (I + II = index). Bushnell and Treuholtz (1908) advocated the summation of the first two and half of the third groups (I + II +

III/2 = index). The latter method is the most used in this country. For the purposes of this paper we have used the second method, more especially because of the greater preponderance of cells in the higher groups in the rat.

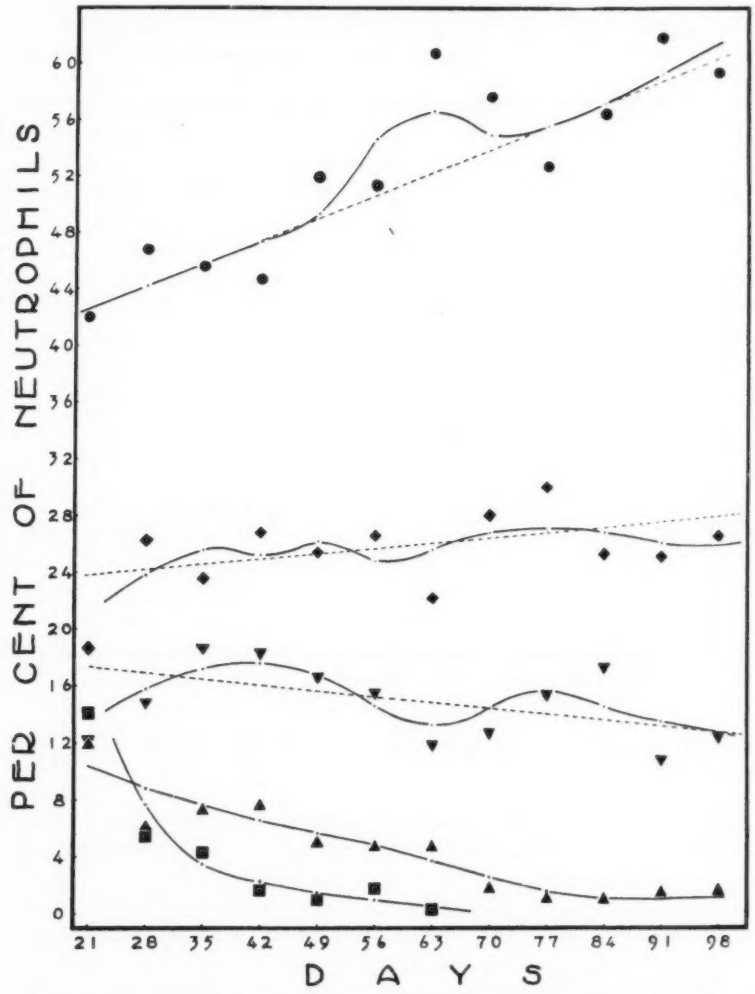

Chart I. Age variations of Arneth neutrophil groups in the rat. Group I. Squares. Group II. Triangles. Group III. Inverted triangles. Group IV. Diamonds. Group V. Round plaques.

METHODS. Seventy-two rats of the pied or hooded type were used in this experiment. These animals were of a highly inbred stock—a crossing of the Wisconsin and Johns Hopkins strains. They weighed uniformly 40 to 50 grams at weaning. These were placed on an adequate fixed diet[2] at the weaning age of 21 days. Red and white blood cell counts (Thoma-improved Neubauer) (Trenner, 1924), together with hemoglobin determinations (Newcomer 1919, 1923), and smears for differential counts were made from tail bleedings. Precautions were observed to avoid

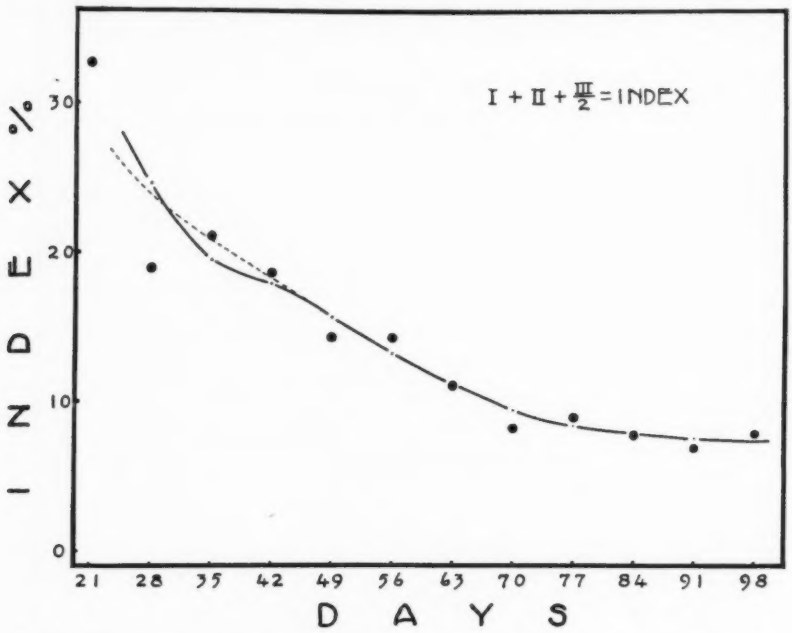

Chart II. Age variations of the Arneth index in the rat. Derived from data of table 1.

excitement, sweating, etc., and the subsequent leucocytosis. Fresh unclipped, healthy animals were used each time. Determinations and

[2]

	per cent
Purified casein	18
Salt mixture (Hawks and Oser (1931)) (Modification of Osborne and Mendel (1917))	4
Wesson oil	4
Cod liver oil	2
Brewer's yeast	8
Corn starch	64

readings were made weekly at the same hour of the day. Five to ten animals were used for each average. Each differential was made from the reading of 600 to 1800 cells (Wright's stain), according to the percentage of neutrophils present. Any marked change in the total neutrophil per cent precluded the discarding of that particular count.

RESULTS. At 21 days the so-called neutrophil cells contain large granules, which show a tendency to be more acidophilic than are those in cells of the more mature animals. A great proportion of the neutrophil nuclei are circinate. The acidophilic tendency presents many border-line cells in classification. At this stage further division of group I, as in the Schilling count, is suggested, but for the purposes of this study we restrict the analysis to the scheme herein presented. At 35 days the neutrophil nuclei show a tendency to early looping and the staining tends to be more basophilic in nature. Marked looping is manifest at 42 days of age. The strands of nuclear material are still full and rounded out at 49 days, but show greater irregularities imposed by the looping. At 63 days the knotted skein-like appearance has developed. At 91 days practically all neutrophil nuclei are well formed in the characteristic tangled skein structure. The group centile averages together with the other blood findings are given in table 1.

In considering the findings on the groups as outlined above we find a definite decrease with age in the cells of group I from 14.1 per cent at 21 days to a total disappearance after 63 days. Group II has a steady, but less abrupt decline, from 12.6 per cent at 21 days to 1.7 per cent at 98 days. Group III varies around 15 per cent with a slight, but not marked, downward trend. A slight centile increase with age is shown by group IV, from 18.3 per cent at 21 days to 26.6 per cent at 98 days. Group V has a more abrupt rise from 42.4 per cent the 21st day to 59.4 per cent the 98th day. The findings for this group show the greatest variation from the mean, whereas groups I and II show the least (chart I). Summation of the first two and one-half of the third groups for the Arneth Index, after the method of Bushnell and Treuholtz (1908), gives a steady decline in the index number from 32.34 per cent at 21 days to 7.82 per cent at 98 days of age (chart II).

DISCUSSION. As will be noted the groups differ from those of the human counts in that a definite progressive centile variation is noted from groups I to V; whereas, in the human counts the peak is generally found in group II or group III, with a recession in groups IV and V. In the above findings groups I and II, and groups IV and V, vary inversely with age; that is, the younger cells decrease as the rat's age increases, while the reverse is true for the older cells. The degree of variation from the mean tends to increase in groups I to V. In view of the theory of the Arneth count, we believe the above method and findings present a more accurate

application of the Arneth groups to the neutrophil blood cells of the rat. The centile variation with age presents a degree of substantiation for the particular choice of criteria in classifying these cells.

SUMMARY

1. Highly inbred rats at various ages on an adequate fixed diet were used for blood differential counts, including the Arneth.

2. The degree of looping, rather than the lobulation of the nucleus is proposed as the criteria for the neutrophil groups in the Arneth count of the rat.

3. The trend with age of the first two or younger groups of neutrophils is downward. The third or mean group fluctuates without a very definite trend. The latter two or older groups show a centile increase, or upward trend.

4. There is a steady decline in the Arneth Index (shift to the right) during the first 98 days of the rat's life.

5. The method offers a more accurate evaluation of the neutrophil count in the experimentally much used rat.

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A STUDY OF THE PITUITARY FACTOR INCREASING THE OVARIAN WEIGHTS OF IMMATURE RATS WHEN INJECTED IN COMBINATION WITH PREGNANCY URINE[1]

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It has been demonstrated that when a growth-free gonadotropic hormone fraction of sheep pituitary is combined with an extract of human urine of pregnancy and injected into sexually immature female rats, the increase in size of the ovaries obtained is greater than the sum of the increases produced when each extract is given separately (Leonard, 1932). This phenomenon has been confirmed and extended by Fevold *et al.* (1933a) and by Evans *et al.* (1933). This intensified gonadotropic stimulation secured when two gonad stimulating hormones are combined has been the outgrowth of the original work of Fevold, Hisaw and Leonard (1931) in which they demonstrated a somewhat similar reaction with combinations of two fractions of the pituitary gland itself.

Evans and his co-workers first reported the administration of a combination of human pregnancy urine with hypophyseal extracts (1932). In this paper they stated that the urinary gonadotropic hormone activated their sex-free growth hormone, converting it into a hormone with gonadotropic properties. They designated this reaction as "activation" and further stated that their pituitary sex-hormone preparation could not be "activated."

Additional data has been secured on the effects of combining pregnancy urine extracts with alkaline extracts of the human hypophysis as well as with similar extracts of the beef, sheep and hog pituitaries. A report of these studies, together with a more extensive account of the early work with pyridine extracts of the sheep A.P., will be given in this paper.

EXPERIMENTAL PROCEDURE. Immature female rats between the ages of 22 and 24 days were used as test animals. In every case, a litter of at least 3 animals was used, one receiving pregnancy urine (P.U.)

[1] Aided in part by grants from the National Research Council, Committee on Problems of Sex, administered by Dr. P. E. Smith.

[2] National Research Fellow.

extract, one, the anterior pituitary fraction (A.P.) and the other the combination of the 2 substances in the same dosage. (Uninjected controls were not used in order to conserve animals.) An ovarian weight of 15 mgm. was taken as the average weight of the untreated control gonads as a basis of calculation. The larger litters were evenly divided into 3 groups as far as possible and the results from each group averaged.

For testing extracts for the presence or absence of the growth hormone, adult hypophysectomized rats and dwarf mice were used. These two types are very sensitive to the growth hormone. I am indebted to Dr. P. E. Smith for the operated animals used in these tests.

In the first experiments, the water soluble fraction of the pyridine extract of sheep A.P. (Fevold, Hisaw and Leonard, 1931) was used in combination with pregnancy urine. This fraction is known as the follicle-stimulating fraction because it is capable of producing ripe follicles in the ovaries of immature rats with accompanying oestrous changes in the accessory organs. The particular preparation used was not as pure as has been made subsequently (Fevold, Hisaw, Hellbaum, Hertz, 1933b) because it caused quite rapid luteinization of the ovaries in the period injected.

The alkaline extracts of the A.P. used in the other experiments were prepared as follows. Five grams of the powdered glands[3] were extracted for 12 hours in the cold with 200 cc. of a 0.2 per cent solution of NaOH. (The human pituitaries were extracted individually using proportionally smaller amounts of solution.) The mash was adjusted to a pH of 8.5 and centrifuged. The supernatant liquid was added to 5 volumes of acetone, centrifuged and the precipitate dried. This was dissolved in a sodium hydroxide solution (pH 8.3) so that 1 cc. equalled 20 mgm. of the original dried gland. The quantities of pituitary extracts employed are expressed in milligrams of the original dried pituitary powder.

Both Follutein (Squibb) and an extract of pregnancy urine, made by the alcohol precipitation method of Zondek (1931) were used.[4] The amounts used are based on rat units or in equivalents of cubic centimeters of the whole urine. The rats were injected subcutaneously with 1 cc. doses daily for 4 days and killed one day after the last injection. At autopsy, the ovaries were weighed and in some cases, serially sectioned.

[3] The hypophyseal material from the pig, sheep, cattle, were furnished through the kindness of Parke, Davis & Co. The human pituitaries were obtained through the courtesy of Dr. R. Kurzrok of the Department of Obstetrics and Gynecology, College of Physicians and Surgeons and Morrisiana Hospital.

[4] The Follutein was presented through the courtesy of Dr. J. A. Morrell of Squibb & Sons.

EXPERIMENTAL. *P.U. extracts combined with pyridine extracts of sheep A.P.* In the first series of experiments, a pregnancy urine (P.U.) extract prepared by the alcohol method was employed. In the combined treatment, this and the sheep A.P. were injected either separately or mixed. In all cases, the total volume of fluid administered was the same. Since the results were similar, whether the two gonadotropic principles were injected separately or mixed, the two substances were united for simplicity of handling in all further experiments. Several batches of hypophyseal extracts were tested.

TABLE 1

Augmentation with P.U. extract and pyridine extract of sheep hypophysis

LITTER	TREATMENT		AVERAGE OVARIAN WEIGHT (MGM.)			AUGMENTATION	REMARKS
	P.U. Ext.*	S.A.P. Ext.**	P.U. treated	S.A.P. treated	Combined treated		
		grams				*per cent*	
GH6343-6	1 cc. = 1 cc.	0.10	(1) 19.0‡	(1) 18.5	(2) 40 5	240	Injected separately
GH6200-4	1 cc. = 5 cc.	0.15	(1) 23.9	(1) 20.0	(3) 83.2	319	Injected separately
GH6190-6	2 cc. = 10 cc.	0.25	(2) 41.6	(2) 20.0	(3) 83.0	115	Mixed before injecting
GH6159-65	1 cc. = 5 cc.	0.15	(2) 24.6	(2) 77.4	(2) 166.1	110	Injected separately
GH6213-8	1 cc. = 5 cc.	0.20	(2) 22.0	(2) 29.0	(2) 86.9	233	Injected separately
G7402-7	1 cc. = 1 cc.	0.08	(2) 13.7	(2) 12.0	(2) 40.0	250	Mixed before injecting

* P.U. extract made by alcohol ppt. method, in terms of original amount of urine.
** Water soluble pyridine fraction.
‡ Numbers in parentheses indicate number of animals.

The amounts of material used and the results obtained by combining P.U. and the water soluble pyridine fraction are given in table 1. With litter GH6200-4, for example, the increase in weight of the ovaries due to P.U. was (23.9 minus 15) = 8.9 mgm.; the increase due to the A.P. factor was (20 minus 15) = 5 mgm. However, when the two substances were combined, instead of obtaining an increase of 13.9 mgm., an increase of (83.2 minus 15) = 68.2 mgm. was secured, or an "augmentation"[5] of 319 per cent over the increase expected.

[5] The term "augmentation" as applied by the Wisconsin group is adopted for the present discussion since it is best suited for the description of the reaction.

Furthermore, in litter GH6159-65 two rats were treated with double the amount of the respective extracts given to their littermates. In neither case did even a doubling of the weight of the ovaries occur. This seems to indicate that there is a fundamental physiological interaction between these two different substances which produces these heavy ovaries.

The augmentation in these experiments could not be due to an activation of the growth hormones, as was first maintained by Evans, Meyer and Simpson (1932), for the most sensitive tests did not reveal the presence of any of this hormone. Tests for the growth hormone were made with hypophysectomized rats (4) and dwarfed mice (4). Injections for periods of 10 to 15 days elicited no growth response whatsoever, but the sex organs were increased in size. It seems that the growth hormone is not essential for augmentation and it can be considered as an interaction of gonadotropic hormones.

The Wisconsin group have recently further purified the follicle-stimulating substance (Fevold *et al.*, 1933b), and also have shown definitely that it is the follicle-stimulating fraction that is involved in augmentation (Fevold *et al.*, 1933a). They have demonstrated that their luteinizing fraction will not give an increased stimulation when combined with P.U. extract. I have confirmed their findings on this point. In one series, for example, the average ovarian weight of the P.U. treated animals was 35 mgm., of the water insoluble fraction (luteinizer) 7.5 mgm., and of the combination 37 mgm.

The question arose as to whether this phenomenon could be demonstrated using glands of other species as sources of the pituitary factor and whether alkaline extracts of the gland would also give augmentation.

P.U. extract combined with alkaline A.P. extracts. Since it was demonstrated that the human anterior hypophysis has properties characteristically different from the urine of pregnant women (Leonard, 1933), it seemed of interest to combine alkaline extracts of this gland with pregnancy urine. When given alone, an alkaline extract of it produced large ovaries in the immature rat even when extracts containing small amounts of the gland were injected (table 2). When given in amounts so small that ovarian weights are not increased, it is still capable of stimulating follicular hormone secretion as evidenced by the enlarged uteri. For example, doses of 1 to 2 mgm. have but a physiological stimulating effect on the ovaries; yet when increased to 4 or 10 mgm., a marked enlargement of the ovaries is produced. The human pituitary is especially rich in the gonad stimulating complex.

However, when varying doses of human pituitary extract were combined with the P.U. extract, augmentation was very difficult to secure (table 2) Indeed, it only occurred when low doses were used,

failing completely on high doses where large ovaries were produced by the pituitary extract alone.[6]

The probability that the extract of human A.P. would give augmentation with sheep A.P. was suggested because of the capacity of pregnancy urine from women to give augmentation. In four litters of animals, doses of 4 mgm. of human A.P. and 23.3 mgm. of sheep A.P. were injected alone and simultaneously. The percentage augmentation resulting was 79, −6, 54, and 27, which seemed to indicate no definite augmentation with such a combination.

In contrast to the human A.P., alkaline extracts of beef, sheep or hog pituitaries combined with pregnancy urine gave marked augmentation

TABLE 2

Augmentation with P.U. extract and an alkaline extract of human hypophysis

LITTER	TREATMENT		AVERAGE OVARIAN WEIGHT (MGM.)			AUGMENTATION
	P.U. Ext. R.U.	Alk. H.A.P.	P.U. treated	A.P. treated	Combined treated	
	*cc.**	*mgm.*				*per cent*
GH8540–44	5	1	(1) 23.0**	(1) 13.5	(3) 32.7	121
GH8535–9	5	1	(1) 24.6	(1) 13.0	(3) 30.5	61
G8565–70	10	2	(2) 32.0	(2) 17.5	(2) 50.8	84
GH7848–51	10	4	(1) 37.0	(1) 38.5	(2) 84.5	53
W7899–901	10	4	(1) 34.0	(1) 35.0	(1) 81.0	69
GH7895–98	10	4	(1) 21.0	(1) 65.0	(2) 63.3	−14
GH8971–74	10	4	(1) 29.8	(1) 86.0	(1) 88.8	−14
G8585–88	10	10	(1) 30.5	(1) 85.5	(2) 93.7	−8
GH8611–13	10	10	(1) 41.5	(1) 184.5	(1) 185.0	−13
B287–89	10	10	(1) 43.0	(1) 105.0	(1) 116.0	−14

* Pregnant blood serum.

** Numbers in parentheses indicate number of animals.

even when large doses were given. When an extract of 20 mgm. of the beef A.P. was combined with 10 R.U. of Follutein some augmentation occurred but when 60 mgm. were used, a constant large increase resulted (table 3). It is seen that relative to the human A.P. extract, larger amounts of bovine hypophyseal extract are needed to increase the ovarian weights of the immature rats when given alone, yet when combined with P.U. extracts, a greater augmentation was obtained at this increased dosage. Furthermore, the alkaline extract of the beef A.P.

[6] Fairly good augmentation has been more recently secured when small doses of P.U. extract and human A.P. extract are combined. In no case did the weights of the ovaries stimulated by the combined extracts exceed 59 mgm., nor when each was injected separately did they exceed 20 mgm.

in the doses given did not stimulate the growth of the uterus which is in marked contrast to the effects produced by the human pituitary.

Results intermediate to those obtained with the human and beef A.P. were secured with alkaline extracts of sheep A.P. (table 4). Ten milligrams of sheep A.P. combined with 10 R.U. of Follutein gave excellent augmentation. With a dose of 23.3 mgm., consistently high augmentation was not secured. This is in accord with the results ob-

TABLE 3

Augmentation with P.U. extract and an alkaline extract of beef hypophysis

LITTER	TREATMENT		AVERAGE OVARIAN WEIGHT (MGM.)			AUGMENTATION
	P.U. Ext. R.U.	Alk. B.A.P.	P.U. treated	A.P. treated	Combined treated	
		mgm.				*per cent*
GH9174–6	10	20	(1) 33.5*	(1) 18.0	(1) 73.5	172
GH9161–7	10	20	(2) 29.8	(2) 11.0	(3) 43.5	92
G9205–10	10	20	(2) 39.5	(1) 16.5	(2) 41.8	3
G9211–3	10	60	(1) 37.5	(1) 24.5	(1) 126.5	238
GH9270–73	10	60	(1) 38.5	(1) 34.5	(2) 122.5	150
GH9259–61	10	60	(1) 34.4	(1) 29.5	(1) 109.0	177

* Numbers in parentheses indicate number of animals.

TABLE 4

Augmentation with P.U. extract and an alkaline extract of sheep hypophysis

LITTER	TREATMENT		AVERAGE OVARIAN WEIGHT (MGM.)			AUGMENTATION
	P.U. Ext. R.U.	Alk. S.A.P.	P.U. treated	A.P. treated	Combined treated	
		mgm.				*per cent*
G8701–4	10	10.0	(1) 25.0*	(1) 12.0	(2) 66.5	415
GH8708–11	10	10.0	(1) 20.5	(1) 15.5	(2) 40.2	320
GH7852–5	10	23.3	(1) 42.5	(1) 22.0	(2) 85.5	104
BH7878–82	10	23.3	(2) 20.0	(1) 13.3	(2) 59.5	799
BH7905–11	10	23.3	(2) 46.8	(2) 15.3	(2) 94.3	147

* Numbers in parentheses indicate number of animals.

tained with the human A.P., though not so marked, in which the phenomenon could not be demonstrated with large doses of the gonadotropic hormone complex.

Hog pituitary extract, when combined with P.U. extract also gave pronounced augmentation. With 10 R.U. of Follutein and 20 mgm. of hog A.P., increases of 83, 114, and 139 per cent were secured in 3 litters of rats.

It can thus be stated that alkaline extracts of beef, sheep and hog

pituitaries give a greater augmenting effect with P.U. extract than does human A.P. It would seem possible by giving graded doses to further classify these four as to their relative ability to give the increased stimulating effect when combined with this urinary gonadotropic hormone.

Augmentation from combined injections of two gonadotropic principles in human urine. It has been shown that a follicle-stimulating hormone from the urine of certain individuals when combined with P.U. also gives augmentation in normal and hypophysectomized rats (Leonard and Smith, 1933a).

A litter of 5 immature hypophysectomized rats after a post-operative period of 5 days were injected for 5 days with an equivalent of 100 cc. of urine containing the follicle-stimulating hormone. At the termination of these injections, four received an intraperitoneal injection of 100 R.U. of Antuitrin S. Two of the latter group were killed 24 hours later, as was also the rat receiving only the follicle-stimulating extract. Forty-eight hours after the Antuitrin S injection, the other two were killed.

The ovaries of the rat treated with follicle-stimulating hormone alone weighed 33 mgm. This weight closely approximated those of 6 other similarly treated hypophysectomized rats. As in the other cases also, the ovaries contained only follicles. The weights of the ovaries of the two killed 24 hours after P.U. treatment were 75 and 54 mgm., the two killed 48 hours after P.U. injections were 113 and 128 mgm. The last two showed beginning formation of corpora lutea. Injections of these 2 urinary gonadotropic principles also give augmentation in normal immature rats (Leonard and Smith, 1933a). The similarity of these results in the normal and hypophysectomized rats will be discussed later.

Discussion. Since it has been possible to demonstrate the synergistic action of pregnancy urine extracts and hypophyseal extracts devoid of growth hormone but containing sex stimulating hormones, the activation of the growth hormone as postulated by Evans (1932) cannot be accepted. The reaction must be considered as involving gonadotropic hormones. Augmentation obtained by using the pyridine extract of sheep hypophysis free of growth hormone has been confirmed by Fevold *et al.*, (1933a) and Evans *et al.* (1933).

Furthermore, it has been established that alkaline extracts of human, beef, pig, and sheep hypophysis will also give augmentation with pregnancy urine extract. The extracts from the pituitaries of these different species, when combined with P.U., do not seem capable of augmenting the size of the ovaries to the same degree. There also appears to be an optimum amount of hypophyseal extract necessary to produce the best results in each case. Augmentation with alkaline extracts of the human

gland was secured only when used in small amounts. On the other hand, it took large amounts of beef gland to give the reaction, the pig and sheep glands requiring amounts somewhat intermediate between the other two. The explanation of this *species difference* in the ability to obtain augmentation cannot be stated. The possibility that too much ovarian stimulation by the hypophyseal factor alone will prevent augmentation does not explain the low result with human A.P. for the limit of response to augmentation is reached before the maximum size ovaries of the animal is reached.

Augmentation as obtained with combinations of the P.U. extract with the urinary follicle-stimulating extracts in normal and hypophysectomized rats might possibly be explained in the following manner. The follicle-stimulating substance sensitizes the ovary (particularly the ovarian follicle) so that a greater response to the luteinizing substance (P.U.) can take place. Our knowledge of the effect of P.U. on the ovary of the hypophysectomized female and normal immature female is more complete than it is with the hypophyseal extracts; it is known that there is a limit to the effect of P.U. extract on the rat ovary (Leonard and Smith, 1933b). By increasing the sensitivity of the ovary, P.U. extract is more effective in producing heavier ovaries of a size greater than that which would be expected from the additive effect of the substances injected individually. Associated with the increase in weight, there is an intense luteinizing action, best observed when the two urinary principles were given together in the normal immature females or in sequence in the hypophysectomized rat.

It is not entirely improbable, in the light of our present knowledge of the gonadotropic hormones, that a substance somewhat similar to that found in the follicle-stimulating urines might be present in the hypophyses of the several species of animals tested. The isolation of this substance from the gland must be accomplished before the above explanation can be applied to all cases of augmentation. However, this suggestion has some experimental backing because the Wisconsin group has obtained augmentation by combining 2 fractions of the sheep hypophysis or by substituting P.U. for one of the fractions, the luteinizer (Fevold *et al.*, 1933a).[7] Likewise, the other fraction, the follicle-stimlator, has some properties similar to those of the follicle-stimulating urines.

The California group (Evans et al., 1933) believe that augmentation is due to the action of P.U. extract with a hitherto unknown fraction of the hypophysis which by itself is inactive and which has chemical prop-

[7] Whether the augmentation secured by the hypophyseal luteinizer and the P.U. extract is based on the same fundamental principle is unknown.

erties different from any fraction yet described. The evidence of this awaits verification.

In conclusion, it is not possible as yet to definitely state the nature of the mechanism of the synergistic action of these 2 hormonal substances. It appears from these experiments that each gonadotropic hormone used has a definite and specific effect on the end organ, so it would seem quite logical to suspect some mutual interaction on the gonad to produce these extra large ovaries. In other words, the ovary itself may play a more important rôle than has been attributed to it in giving this synergistic effect.[8]

SUMMARY

1. The increase in weight of the ovaries of immature rats produced by the combined injections of pregnancy urine and growth-free gonadotropic hypophyseal extracts is greater than can be predicted by adding the increases produced by each extract separately.

2. This synergistic action can also be demonstrated by combining alkaline extracts of the sheep, beef, hog and to some extent, human pituitaries, with pregnancy urine extract.

3. There is not a uniform degree of augmentation when hypophyseal extracts from several different species are used.

4. Human pituitary extract failed to give augmentation when given in large doses and gave only a moderate degree of augmentation with low doses.

5. By injecting a follicle-stimulating hormone prepared from urine into hypophysectomized rats and following this treatment with pregnancy urine injections, a rapid increase in the weight of the ovaries results with a luteinization of the follicles. It seems plausible that the synergistic reaction of these combined injections is dependent on a differential action of 2 gonadotropic hormones which produce a greatly enlarged ovary.

[8] Since this manuscript was prepared, another series of articles concerning augmentation has appeared from the California laboratory. In their series of papers they have been consistently shifting their viewpoint and repudiating their previous conclusions. They first stated that the growth hormone was converted by pregnancy urine into *the gonadotropic* hormone. Later (Evans *et al.*, 1933) they state that the hypophyseal factor giving augmentation was a separate inactive pituitary factor, free from the growth hormone and the gonadotropic hormone. Finally, they have stated that the synergic pituitary factor is active by itself but insist that this activity is different from that of two other gonadotropic hormones (thus recognizing that there are three). It does not seem that their evidence conclusively establishes a difference between the follicle-stimulating and the synergic factor (Evans *et al.*, 1933, Journ. Exp. Med., **58**, 550).

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THE SOURCES OF ENERGY IN MUSCULAR WORK PERFORMED IN ANAEROBIC CONDITIONS

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In previous papers we have shown that the lactic acid removal during recovery from muscular exercise does not fully account for the payment of the oxygen debt: an "alactacid" mechanism of contracting and paying the oxygen debt was detected which goes at a much faster rate than the "lactacid" mechanism, and which is responsible for about one-third of the total oxygen debt (Margaria, Edwards and Dill, 1933; Margaria and Edwards, 1934.)

Prof. A. V. Hill suggested to us that the existence of the two mechanisms may be shown in the following way: according to our previous work, in five minutes the alactacid oxygen debt is completely paid, while in that time only a small fraction of the payment of the lactacid oxygen debt can take place; also it has been known for a long time that, after short strenuous exercise, the lactic acid concentration in the blood may increase to very nearly the maximum values. Then, allowing a five-minute period of rest between two short and strenuous runs, performed in practically anaerobic conditions, if in the first run the subject exhausted his lactacid mechanism for contracting oxygen debt, the second run will be performed nearly exclusively at the expense of the alactacid mechanism. In this way the existence of the alactacid mechanism for contracting oxygen debt could be detected, and its relative importance evaluated. On these lines we devised the experiments described in the present paper.

METHOD. Four experiments were performed on a man, aged 22, weight 63.0 kgm., untrained to hard exercise. The subject was previously trained to run on the treadmill and to jump on and off. The treadmill was set at a speed of 18.7 km. p. hour and at an 11.5 per cent grade. The subject jumped on the treadmill and ran until exhausted; the time of the performance was recorded. At the end of the run he sat in a chair while blood samples were drawn from the arm vein for lactic acid determinations and

[1] It is a great pleasure to acknowledge here my indebtedness to Prof. L. J. Henderson for his interest and his support of this work and of the ones previously published: also to him and to the other members of the Fatigue Laboratory for their kindness and hospitality, for all the possibilities for work and for the collaboration, of which I have benefited during my stay at the Fatigue Laboratory. R. M.

the expired air collected for analysis. After 5–6 minutes of rest, the performance was repeated in the same way, and a third time again after 5–6 minutes from the second performance. The duration of the running periods as well as the duration of the rest periods are recorded in the protocols. (See figs. 1 to 4.)

RESULTS. *Relation between amount of work done and increase of lactic acid concentration in the blood.* In figure 5 the difference for each period of exercise in every experiment between the highest value of concentration

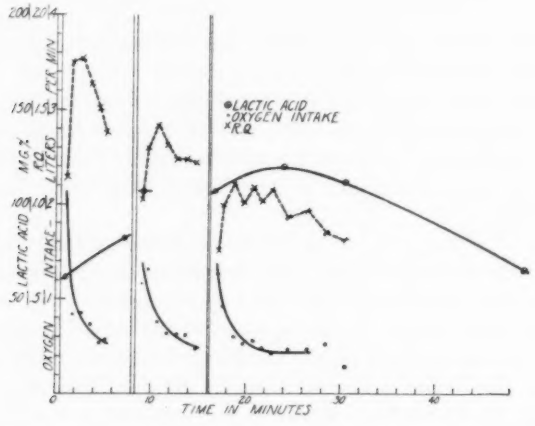

Fig. 1. Oxygen consumption, respiratory quotient, and lactic acid concentration in the blood as functions of the time since the beginning of the first run. The periods of the runs are indicated by the three pairs of vertical lines starting at the corresponding times shown on the abscissa.

Experiment of September 20, 1933. The three periods of run lasted respectively 32, 25 and 15 seconds.

Lactic acid values: beginning and end of first recovery period, respectively *62.6* and *82.2;* beginning of second recovery period: *107;* third recovery period: 17¼, *107;* 24, *120;* 30, *112;* 50, *65.7.* The figures in roman represent the time in minutes from the beginning of the first run, the figures in italics represent the lactic acid values.

of lactic acid in the blood in the 5 minutes following the period and the concentration of lactic acid immediately before the period is plotted against the time of the duration of the run, for each of the three periods of each experiment.

The following assumptions are made: *a.* The duration of the run is proportional to the amount of work done, the extra amount of work due to jumping on the treadmill being neglected. *b.* The amount of oxygen used up during the work is very small as compared with the amount of oxygen

consumed during recovery (about 10 per cent for the first run): it may be considered without a serious error, proportional to the time of running. Therefore *c*, the duration of the run is proportional to the amount of work performed anaerobically. *d*. This anaerobic work being proportional to the oxygen debt, the duration of the exercise is proportional to the

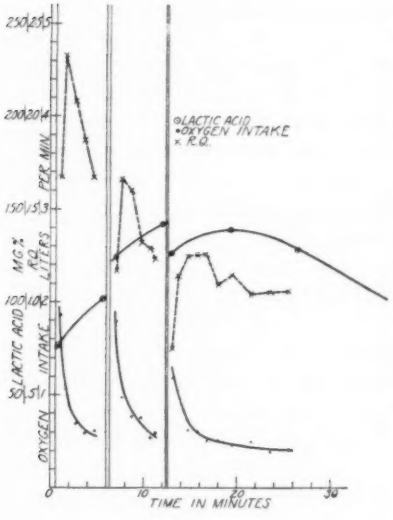

Fig. 2

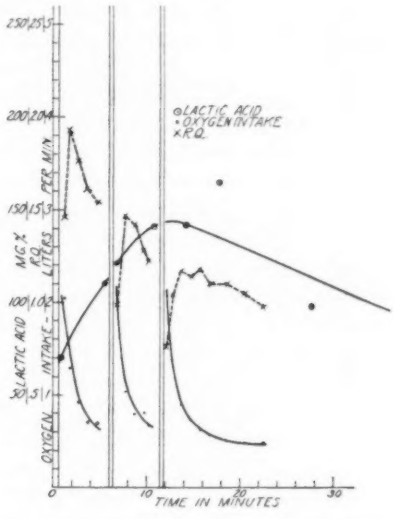

Fig. 3

Fig. 2. Experiment of September 26, 1933. The three periods of run lasted respectively, 34, 23 and 15 seconds.

Lactic acid values: first recovery period: beginning, *77;* end, *102;* second recovery period: beginning, *124;* end, *142;* third recovery period: beginning *136;* 19¼, *139;* 26½, *128;* 43, *90.4*.

Fig. 3. Experiment of September 28, 1933. The three periods of run lasted respectively, 36, 20 and 16 seconds.

Lactic acid values: 55″, *70;* 5½, *110;* 6′50″, *121;* 10′45″, *141;* 14′15″, *142;* 17′45″, (*165*) 27′30″, (*98*); 42′30″, *90;* 61, *55.5*. The figures in brackets are not very reliable being determined with only 1 cc. of blood which was obtained with difficulty from the arm vein, and only after stasis. They were not taken into account in the discussion of the data in the paper; they would not, however, alter the final conclusions.

oxygen debt. *e*. The breaking down of glycogen into lactic acid is one of the sources for anaerobic work. *f*. The difference in blood lactic acid concentration (cf. fig. 5) is proportional to the amount of lactic acid formed during exercise: as the lactic acid data represent the condition at 5 minutes after the end of the work, the error due to time for diffusion from muscles to blood is greatly reduced; another error may be due to the

oxidative removal of part of the lactic acid during this time.[2] On the other hand an error in the opposite direction may set in, which consists in the delayed lactic acid production.

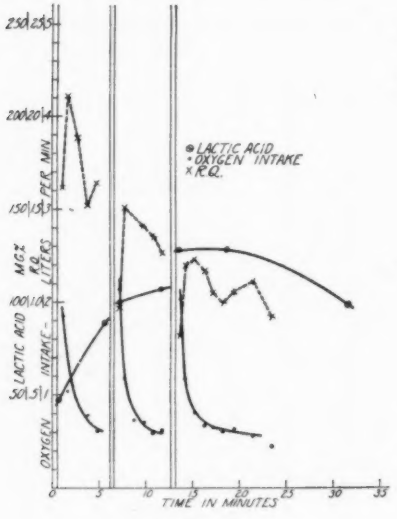

Fig. 4

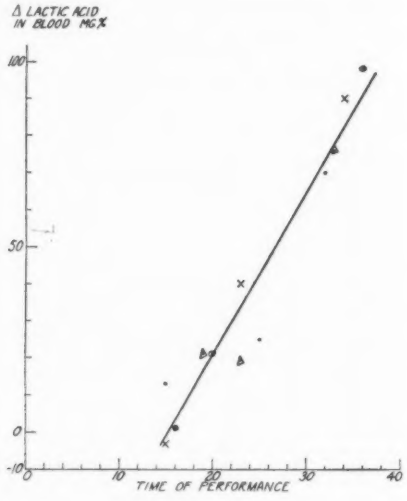

Fig. 5

Fig. 4. Experiment of October 4, 1933. The three periods of run lasted respectively 33, 23 and 19 seconds.

Lactic acid values: 50″, *47.3;* 5′40″, *88.4;* 7′10″, *100;* 11′45″, *107;* 13′25″, *128;* 18′45″, *128;* 31′40″, *99;* 45′45″, *77.4;* 62′, *47.3.*

Fig. 5. Difference in blood lactic acid concentration (mgm. per 100 cc.) as a function of the time of performance of work. See text.

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[2] In the first few minutes of recovery the lactic acid removal from blood in man shows a delay that may be interpreted as due partly to slowness of diffusion from muscles to blood, partly to delayed lactic acid production, and partly to a slower oxidative removal of the lactic acid itself. It is not possible to evaluate quantitatively the part played by these single factors separately: however the experiments on mice (Margaria and Edwards, 1934) where only the last two factors were involved seemed to show that for a few minutes after exercise the oxidative removal of lactic acid goes at a slower rate than it does later.

The maximum error due to oxidative removal of lactic acid may be calculated if the assumption is made that the removal of lactic acid is of a logarithmic character, and that its velocity may therefore be calculated from that observed after the first say 10 minutes of recovery (velocity constant = 0.02). Then if the extralactic acid concentration in the blood is 100 mgm. per 100 cc., the removal of lactic acid would correspond to 4 mgm. per minute, and in the 4 to 5 minutes of observation, to a difference of 20 mgm. per 100 cc.

It may be seen in the following figure 5 that increasing all the values of the ordinates by 20, the value of t_1 for L. A. = O would be 4.5 seconds less than the value given. This maximum possible error does not affect the conclusions that are given later.

On these assumptions the relationship in figure 5 has to be a linear one, as it appears to be experimentally, with a fairly good approximation; as calculated with the method of least squares, it is defined by the equation:

$$\Delta \text{ L. A.} = -65.9 + 4.33t$$

From such a relationship the following conclusions are drawn:

a'. Because it is linear, it appears very probable that the process of lactic acid formation from glycogen is really one of the sources of energy for anaerobic work, thus supporting assumption *e*.

b'. The fact that the line cuts the abscissa at $t = 15$, indicates that the lactic acid mechanism is not the only one to account for the performance of anaerobic work, in which case for L. A. = 0, t should also be zero. The probable error of L. A. has been calculated as $\pm$ 6.1 (probable error of t is $\pm$ 1.4): the statistical probability that $t = 0$ for L. A. = 0 is therefore zero. There seems to be another mechanism which accounts for as much energy as would be produced by an extra production of 66 mgm. of lactic acid per 100 cc. of blood. Furthermore this mechanism is fully restored after a five minute rest.

These results cannot be explained on the hypothesis that lactic acid formation is the only mechanism for contraction of an oxygen debt. In fact, in our experiments the third run was performed in anaerobic conditions as well as the first, but the accompanying lactic acid production was trifling.

Assuming a uniform distribution of lactic acid through the body, and assuming that the water content of the blood is 80 per cent, and that the water content of the whole body is 72 per cent, the weight of the subject being 63 kgm., 100 mgm. of lactic acid per 100 cc. of blood corresponds to 56.6 grams in the whole body. The formation of 1 gram of lactic acid from glycogen, in the muscles, liberates 0.340 Calorie. Therefore the amount of anaerobic heat dissipated in exercise corresponding to the liberation of 66 mgm. lactic acid per 100 cc., the figure arrived at above as representative of the energy production of the alactacid mechanism, amounts to about 13 Calories. This conclusion, on the assumption that the mechanical efficiency of this mechanism is the same as for the lactacid mechanism, corresponds to 2.6 liters of oxygen. Assuming that the efficiency of the oxidative recovery process is say from 80 to 90 per cent, the fraction of the oxygen debt due to the alactacid process would amount to about 3 liters.

Assuming that the maximum increase of lactic acid in the blood as a consequence of muscular exercise is approximately 130 mgm. per 100 cc., the relative amount of alactacid oxygen debt to the total is 1:3.

The magnitude of both the absolute and the relative figures of the amount of alactacid oxygen debt, as calculated above from the amount of

possible anaerobic work, are in close enough agreement with those found on the subject Clapham on quite different grounds; i.e., from the analysis of the curve of the oxygen debt payment (Margaria, Edwards and Dill, 1933). It was shown in that paper that the breakdown of phosphocreatine, which is known to occur during muscular contraction, may be a possible mechanism of contracting the alactacid oxygen debt: the same interpretation applies to these findings.

Thus the performance of work under anaerobic conditions without involving the formation of lactic acid, is demonstrated to take place in man, in perfectly normal conditions, independently of iodoacetic poisoning.

The relation given in figure 5, where it is shown that about one-third of the maximum anaerobic work may be performed independently of the production of lactic acid, does not imply that the lactacid mechanism of performing anaerobic work only enters into play after the alactacid mechanism is exhausted; for example, it is improbable that if the first run were of 15 seconds we should not have found any increase of lactic acid concentration in the blood. We believe that the lactic acid mechanism starts much before the alactacid is exhausted: this we are going to test in further experiments.

The payment of the oxygen debt. This ought to occur approximately with the same modalities and to the same extent in the three periods of recovery. Only the oxygen consumption during recovery ought to be a little higher after the second run, and still a little more after the third because the lactic acid concentration is higher than after the first run (about 25 and 35 mgm. per 100 cc. of blood). A further increase of 30 mgm. above 100 mgm. of lactic acid per 100 cc. of blood, according to our previous paper (Margaria, Edwards and Dill, 1933), would increase the oxygen consumption per minute by about 35 cc.

In figure 6 the oxygen consumptions in excess of the basal oxygen consumption in the three recovery processes in the four experiments are plotted against time for the first few minutes of recovery. On the average, the oxygen consumption seems to be less after the third run than after the first; this in spite of the fact that the lactic acid concentration being higher after the third run, the oxygen consumption ought to be correspondingly higher, if there were no other factors in operation.

This phenomenon may be related to the delay in the removal of the lactic acid that we have observed also in previous experiments after severe exercise. Delayed lactic acid production and slowness of lactic acid diffusion from muscles to blood appear to be insufficient to account for the facts. The delay in the lactic acid removal after the third run in the present experiments is much higher than any previously recorded after a single run.

It seems therefore possible that the oxidative removal of lactic acid is

diminished after severe exercise: this hypothesis would explain both the delay in the lactic acid removal and the lower oxygen consumption in the third recovery, in our experiments.

We do not hazard any definitive hypothesis as to the delay in the oxidative removal of lactic acid after very hard exercise. Modification of the blood circulation in the muscles during recovery may play an important part. Such a delay has been found to be of a particularly high magnitude on untrained subjects: while in trained athletes the rate of lactic acid removal has reached a constant value that is maintained up to the end after only 6 to 7 minutes of recovery, in untrained subjects such a value is not reached half an hour or more after the end of the exercise.

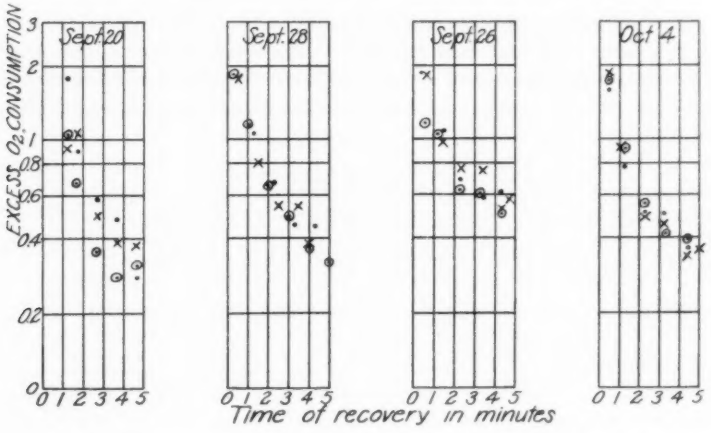

Fig. 6. Oxygen consumption during the three recovery periods, as a function of time of recovery, in the four experiments. The ordinates are plotted on a logarithmic scale. ● 1st recovery period. x 2nd recovery period. ⊙ 3rd recovery period.

We wish to acknowledge our indebtedness to Prof. A. V. Hill for his kind criticism of this paper before publication.

SUMMARY

1. The increase in lactic acid concentration in the blood following muscular work performed in anaerobic conditions is a linear function of the amount of work, as defined by the relation:

$$\text{L. A.} = -a + bW$$

2. As the amount of work performed anaerobically may be considered to be proportional to the oxygen debt, we have:

$$\text{L. A.} = -a' + b'O_d$$

3. The fact that this relation is linear suggests that the breakdown of glycogen into lactic acid is really one of the mechanisms for contracting an oxygen debt, and therefore for performing work anaerobically.

4. The fact that the constant a is negative supports the hypothesis of the existence of a second mechanism for performing work anaerobically, independently of lactic acid formation. This alactacid mechanism is restored in less than 5 minutes. The value of $-a$ is about half the maximum value of L. A.; this means that the amount of work performed at the expense of the alactacid mechanism is about half the amount of work attributable to the lactacid mechanism. These figures are in agreement with previous findings, where the lactic acid produced was related to the amount of oxygen debt and with its speed of payment.

5. The oxygen consumption during the first 5 minutes of recovery is slightly lower after the third run than after the first run. This, together with the slower removal of lactic acid from the blood after severe or repeated exercise, suggests a slower oxidative removal of lactic acid in such conditions.

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BLOOD VOLUME AND HEMATOCRIT DETERMINATIONS IN RABBITS BEFORE AND DURING EMOTIONAL EXCITEMENT

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In previous researches from this laboratory it was found that emotional excitement (anger, fear and pain) produces a marked increase in the specific gravity (Nice, Lindsay and Katz, 1933) and viscosity (Nice and Fishman, 1934) of the blood as well as a marked concentration in the common blood constituents (Katz and Nice, 1934). In order to determine whether these changes are the result of a diffusion of plasma from the circulation into the peripheral tissues or the result of actual tissue breakdown and the inpouring of the products into the blood stream, we have investigated the blood volume and hematocrit changes in emotionally excited rabbits.

The investigations of Binet (1927), Cannon (1929) and Barcroft (1930) have shown that emotional excitement is associated with an augmented epinephrine secretion and a contraction of the spleen. Lamson (1915) Izquierdo and Cannon (1928) and Binet, Cardot and Fournier (1928) found that injections of adrenalin caused polycythemia.

Cruickshank (1926) found that splenic contraction resulting from splanchnic stimulation caused an increase in the blood volume of dogs. Brednow (1931) injected adrenalin intravenously and subcutaneously and found an increase in erythrocytes in the blood stream which was accompanied by a decrease in the circulating plasma. Emotional polycythemia was demonstrated by Lamson (1915), Izquierdo and Cannon (1928) with cats, Barcroft (1930) with dogs, and by Nice, Morris and Elhardt (1930) with rats.

The work of Estrado (1930) and of Swingle and co-workers (1933) shows that a reduced blood volume occurs after adrenalectomy.

PROCEDURE. Healthy adult rabbits ranging between 1543 and 2923 grams in weight were used in these experiments. Both sexes were represented about equally. These animals were given the regular diet of this laboratory. All rabbits were deprived of food and water 16 to 24 hours previous to the experiment.

The blood volume was determined repeatedly by the micro-method of Went and Drinker (1929) with slight modification. The capillary colori-

metric scale was prepared by diluting a one per cent solution of vital red with rabbit plasma in dilutions ranging from 0.1 to 2 per cent instead of the combination of cat, horse and guinea pig serum used by Went and Drinker (1929). Each dilution was put in a capillary tube, the ends sealed with wax, and all tubes fixed respectively onto a white cardboard.

The capillary tubes used in the preparation of the colorimetric scale as well as in the collection of all blood samples and in the determination of the plasma-cell ratio were prepared from glass tubing of a uniform bore with a 0.6 mm. inside diameter and cut into pieces 10 cm. long. A colorless 0.5 per cent heparin solution was drawn through the capillary tubes used for blood collection and dried.

All of our blood volume determinations were made on unanesthetized rabbits. One-tenth cubic centimeter of 1 per cent vital red per 100 grams of animal was injected into an ear vein after the method of Rous (1918). Both ears had been shaved previously and the blood vessels dilated somewhat by warming the ear with an electric bulb. Thus the blood vessels were in a state of vasodilatation and active circulation at the time the dye was injected and the blood specimens withdrawn.

Ten minutes after determining the normal blood volume, the rabbit was fastened back downwards to an animal holder and excited for about three minutes by being teased from time to time with a weak interrupted electrical current using an Harvard inductorium. The animal exhibited all signs of sympathetic stimulation. About three minutes after this excitation the blood volume was again determined. A uniform routine method of dye injection, collection of blood and examination of these samples was followed. The blood samples were always obtained in duplicate, one from each ear, in order to determine the thoroughness of the distribution and mixing of the dye.

The per cent of blood volume to body weight was calculated by taking the average blood volume of the normal state, multiplying by 1.06 (specific gravity of blood) and dividing by the animal's weight.

The following protocol indicates the course of a typical experiment:

Experiment 8. November 21, 1932. Rabbit E-Male. Weight 2017 grams.
9:00 a.m. Sample I-N taken from right ear.
9:03 a.m. 0.87 cc. of 1 per cent dye solution injected into the marginal ear vein of the right ear.
9:07 a.m. Samples II-N taken from both ears.
9:15 a.m. Animal tied to animal holder. Stimulated with induction shocks.
9:19 a.m. Sample I-E taken from left ear.
9:22 a.m. 1.2 cc. of 1 per cent dye solution injected into marginal vein of left ear.
9:25½ a.m. Samples II-E taken from both ears.
9:28 a.m. Centrifugalization of capillary tubes began.
10:25 a.m. Centrifugalization of capillary tubes ends.

	NORMAL STATE	EXCITED STATE
Dye injected	0.87 cc.	1.2 cc.
Plasma corpuscles	80 mm.	88 mm.
Plasma column	55.9 mm.	57.8 mm.
Scale reading	0.60	0.87
Plasma volume	$\frac{0.87 \times 100}{0.60} = 145.0$	$\frac{1.2 \times 100}{0.87} = 137.9$
Plasma per cent	$\frac{55.9 \times 100}{80} = 69.8$	$\frac{57.8 \times 100}{88} = 65.7$
Total blood volume	$\frac{145.0 \times 100}{69.8} = 207.7$	$\frac{137.9 \times 100}{65.7} = 209.4$
Cell volume	$207.7 - 145.0 = 62.7$	$209.4 - 137.9 = 71.5$

RESULTS. The results obtained in 16 experiments on eleven rabbits—six males and five females—are recorded in table 1. The average blood volume in the normal state was 200.8 cc. in comparison to 204.3 cc. after excitement which is an increase of 1.7 per cent. The plasma volume in the normal state was 129.4 cc. and 118.4 cc. after emotional excitement. This is a decrease of 8.5 per cent. In the normal state the corpuscle volume was 71.4 cc. and in the excited state 85.9 cc., an increase of 20.2 per cent.

The average plasma level in the quiet state was 63.8 per cent while in the excited state it fell to 57.7 per cent. The average per cent of corpuscles in the normal state was 36.2 per cent; in the excited state it increased to 42.4 per cent.

The total blood volume averaged 9.6 per cent of the body weight with a range varying between a minimum of 8.2 per cent and a maximum of 11.2 per cent.

DISCUSSION. The blood volume average of 9.6 per cent of the body weight is somewhat higher than the values reported for the rabbit by Went and Drinker (1929) and Erlanger (1921). Although our average is higher, the range of 8.2 and 11.2 is less and falls entirely within the range of 7.1 and 11.7 found by Went and Drinker (1929) by the dye method. The blood volume data reported by these authors were obtained on animals in the anesthetized and operated condition. Our animals have not been subject to any anesthesia or operative procedure. Inasmuch as operative procedures cause gross shifts in the circulating blood volume, we believe that our average of 9.6 per cent of body weight probably approximates the actual value.

The increase in the corpuscular volume in emotional excitement is attributed to splenic contraction with the consequent discharge of erythrocytes into the circulation. This is in accordance with the work of Binet (1927), Barcroft (1930) and Brednow (1931).

TABLE 1

Blood volume and hematocrit determinations in rabbits before and during emotional excitement

	EXPERIMENT NO.	RABBIT NO.	SEX	WEIGHT	TOTAL BLOOD VOLUME	PLASMA VOLUME	PLASMA	CORPUSCLE VOLUME	CORPUSCLES	BLOOD VOLUME IN BODY WEIGHT
				grams	*cc.*	*cc.*	*per cent*	*cc.*	*per cent*	*per cent*
Q*	1	A_1	F	2050	198.7	124.3	62.4	74.4	37.6	10.3
E*					202.0	115.2	57.1	86.8	42.9	
Q	2	B_1	F	2360	253.8	165.1	64.3	88.7	35.7	11.4
E					247.3	131.5	53.2	115.8	46.8	
Q	3	B_2	F	2360	248.5	174.0	61.9	74.5	39.1	11.1
E					249.0	146.3	58.8	102.7	41.2	
Q	4	C_1	M	1937	163.8	107.4	65.8	56.4	34.2	8.8
E					187.9	112.7	60.0	75.2	40.0	
Q	5	C_2	M	1937	159.7	100.0	62.7	59.7	37.3	8.7
E					160.8	92.8	57.8	68.0	42.2	
Q	6	D_1	M	1543	145.5	97.5	67.2	48.0	32.8	9.9
E					145.5	79.8	54.8	65.7	45.2	
Q	7	D_2	M	1543	143.7	94.5	65.7	49.2	34.3	9.8
E					145.3	87.3	60.0	58.0	40.0	
Q	8	E	M	2017	207.7	145.0	69.8	62.7	30.6	10.9
E					209.4	137.9	65.7	71.5	34.3	
Q	9	F_1	F	2130	204.8	143.1	69.9	61.7	30.1	10.2
E					202.0	141.4	70.0	60.6	30.0	
Q	10	G_1	F	2448	197.0	118.3	59.9	78.7	40.1	8.5
E					193.7	118.2	61.0	75.5	39.0	
Q	11	G_2	F	2448	194.3	109.4	56.4	84.9	44.6	8.4
E					184.1	95.6	51.9	88.5	48.1	
Q	12	H	M	2923	243.0	157.9	65.0	85.1	35.0	8.2
E					257.4	141.5	55.0	115.9	45.0	
Q	13	I_1	F	2300	207.3	128.2	62.3	79.1	37.7	9.6
E					214.7	109.4	50.7	105.3	49.3	
Q	14	I_2	F	2300	212.3	127.3	60.0	85.1	40.0	9.7
E					218.7	120.0	54.7	98.7	45.3	
Q	15	J_1	M	2500	197.4	120.2	60.8	77.2	39.2	8.4
E					196.3	107.1	54.5	89.2	45.5	
Q	16	K_1	M	2900	234.3	160.7	68.5	73.6	31.5	8.6
E					255.0	140.5	59.0	104.5	41.0	
Averages				2228	200.8	129.4	63.8	71.4	36.2	9.6
					204.3	118.4	57.7	85.9	42.4	
Per cent differences					1.7	−8.5	−6.1	20.2	6.2	

* Q—Quiet; E—excited.

The mechanism involved in the withdrawal of plasma from the circulation is not clearly understood. In the light of the work of Roberts and Crandall (1933) on the rôle of the portal system in the regulation of the circulating blood volume, there is a possibility that there is a redistribution of plasma during excitement. A considerable amount of plasma may be isolated in the portal system that is not mixed with the dye in the course of several minutes.

Krogh (1929) wrote "The portal system, comprising the capillaries, venules and veins of the stomach, intestine, and spleen together with the portal trunk itself, acts as a variable reservoir of blood in virtue of the double set of resistances with which it is provided." In view of the fact that our results indicate a decrease in plasma volume and an increase in cell volume during excitement it is possible that this "variable reservoir" may withdraw plasma and eject corpuscles into the active circulation during excitement. The incoming erythrocytes apparently tend to compensate for the loss in plasma with the consequent maintenance of the circulating blood volume in a fairly constant state.

SUMMARY

1. This study was undertaken in order to determine the blood volume, plasma volume, and the erythrocyte volume changes in the rabbit during emotional excitement.

2. The total blood volume remained fairly constant and did not show a marked change during the course of excitement.

3. There was a slight withdrawal of plasma from the active circulation during excitement.

4. The erythrocyte volume increased during emotional excitement. This seemed to compensate for the plasma volume decrease in the maintenance of a fairly constant total blood volume.

5. The results are interpreted as indicative of a slight shift of water into the tissues, and a simultaneous inpouring into the blood stream of erythrocytes and the products of tissue breakdown. These factors combined account for the specific gravity and viscosity increase of excited blood.

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THE TUBULAR ELIMINATION OF PHENOL RED IN THE RABBIT KIDNEY

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Evidence from many indirect sources indicates that phenol red is eliminated by the proximal convolution of the mammalian kidney. This report includes more direct evidence supplementing the earlier work.

Marshall and Vickers (1923) showed that 1, in dogs not eliminating urine after spinal section, there is an accumulation of intravenously injected phenol red in the cortex; 2, not all the phenol red in the blood exists in a filterable form; 3, the quantity of unbound phenol red in the plasma is not sufficient to account for the amount found in the urine even if all the plasma could be assumed to be filtered; and 4, maximum elimination does not correspond with the period of greatest blood concentration, but occurs some time later. These findings were confirmed by Marshall and Crane (1924), who demonstrated 1, that concentration of phenol red takes place in the cortex of the secreting rabbit's kidney also, and 2, that the efficiency of the dog's kidney for eliminating phenol red is greatest at lower plasma concentrations, and decreases with higher blood concentrations. Edwards and Marshall (1924) observed phenol red in the cells of the tubules of the cortex corticis in the living rat. Marshall (1931) showed 1, again that filtration alone is insufficient to explain the elimination of phenol red because of the impossible order of blood flow necessary for this process, and 2, that more phenol red is removed from the arterial blood going to the kidney than is filterable from the plasma. Filtration alone is insufficient to explain these results and secretion was invoked to account for the storage of phenol red by the tubules, its delayed elimination and the greater efficiency of the kidney at lower blood concentrations.

Starling and Verney (1925) repeated and confirmed the work of Marshall and Vickers with heart-lung-kidney preparations perfused with defibrinated blood to which phenol red was added. In those cases in which cyanide was added to the perfusion fluid in order to destroy the activity of the tubules, the concentration of phenol red in the "urine" rapidly fell and was reduced to zero apparently when the stored phenol red had been washed out. They also invoked secretion to explain their results.

Richards and Barnwell (1927) suggested that the facts could be equally

well explained by glomerular filtration supplemented by some tubular diffusion. The interpretation of Marshall and his co-workers has been questioned also by Ekehorn (1930) and by McKay and Oliver (1930).

Chambers and Kempton (1933) found that when small pieces of the mouse kidney were explanted in tissue culture and phenol red was added to the nutrient medium, the cells of a few small segments of the proximal convolutions took up the substance, concentrated it in the cells and eliminated it into the lumen. They admitted they were dealing with surviving tissue under different environmental conditions.

METHOD AND MATERIAL. Rabbits were used entirely. Except when otherwise stated, they were injected intravenously with the standard solution of phenol red supplied by Hynson, Westcott and Dunning, in doses of 5 to 6 mgm. per kilo body weight. At various intervals, the rabbits were killed by a blow on the head, the kidney was rapidly removed and thin slices were frozen by immersion in liquid air. The subsequent dehydration in a vacuum at low temperatures was affected by the Altmann freezing-drying method (Gersh, 1932).[1] After the pieces of kidney were dried, they were embedded directly in paraffin in a vacuum, sectioned at 10μ, and mounted dry on the slide. The sections were passed through petroleum ether to remove the paraffin, and dried in the air over a small flame. They were observed directly with the microscope while a stream of anhydrous dimethylamine was passing over the sections. The amine forms with the moisture of the air a hydroxide in sufficient concentration to convert very small traces of phenol red to its alkaline red form. The phenol red present in the kidney is retained in its original site during the freezing and drying, and is insoluble in paraffin and petroleum ether. The trace of water usually present in the atmosphere is insufficient to cause a significant shift in distribution of phenol red. In this way, sections of the kidney are made available for study with no appreciable diffusion of phenol red.

The proximal convolutions were identified in several sections as those containing phenol red by two procedures: 1. All but the few yellow-stained tubules were teased away on the slide while the paraffin remained in the section and mounted through xylol and balsam—these contained a brush border. 2. After deparaffining the section with petroleum ether and treating with dimethylamine, all but a few tubules which were red were removed by teasing, the remaining few being mounted in balsam and studied for a

[1] The operation of the apparatus originally used for this purpose has been considerably simplified by several minor changes. 1. Drying is speeded up by the use of a three-stage mercury vapor pump. 2. The phosphorus pentoxide is placed in a glass tray which just fits into the phosphorus pentoxide chamber, thus facilitating its replacement with the dry powder. 3. The ammonia freezing unit is replaced by a simple glass tube suspended in an electric refrigerator which maintains its temperature automatically at −21°C. to −23°C.

brush border. In addition the terminal portions of the convolution could easily be identified without any dissection, as also the proximal convolution in the cortex corticis.

Most of the rabbits were stock animals used to determine the "normal" course of events in the elimination of phenol red. Some were dehydrated in order to concentrate the urine. Several rabbits were treated as described in earlier expeiments (Gersh and Stieglitz) in order to reduce their blood pressure below the osmotic pressure of the blood colloids and were then injected with phenol red.

RESULTS. *Normal rabbits injected with phenol red.* One minute after the end of the injection, phenol red is present diffusely in the cells of the proximal convolution. The plasma in the veins and arteries and the glomeruli also shows a faint pink flush, although there is no phenol red in the glomerular space itself. Four minutes later, the picture is essentially unchanged. Ten minutes after the end of the injection, only a small amount of phenol red is present in the cytoplasm of the cells of the proximal convolutions; most of the phenol red is in the lumen of the loops of Henle and the collecting ducts, where it is more concentrated. The plasma and renal corpuscles do not contain any visible phenol red. Twenty minutes later, only traces of phenol red are present in the cells of the proximal convolution. A few ducts contain appreciable quantities in their lumen. The picture is essentially the same in the proximal convolution one hour after the end of the injection, but fewer ducts contain phenol red.

The kidney of the dehydrated rabbits is similar to that of the normal, although in all cases somewhat more phenol red is found in the cells of the proximal convolution at the same time interval after the injection.

The kidneys of rabbits injected with *large doses* of phenol red in the form of the sodium salt (0.1 g. per kilo) and killed three minutes after the end of the injection are essentially similar to those of normal rabbits injected with the smaller dose. The proximal convolutions are deep red. The blood plasma in the blood vessels and the glomeruli is red, but the glomerular space is free of phenol red.

Low blood pressure rabbits. By cutting the cord and applying artificial respiration to five rabbits, the blood pressure was reduced and maintained at 20 to 30 mm. Hg for periods of 15 to 60 minutes after the injection of phenol red. In all cases phenol red can not be identified in the glomerular space, the loops of Henle, or the collecting ducts. It is present in gradually increasing amounts in the cells of the proximal convolutions. In one case, it can be definitely identified in the lumen of this portion of the nephron.

Attempts were made to induce storage of phenol red by the convoluted tubules. Repeated subcutaneous or intravenous injections of the substance over long periods of time, followed by removal and freezing of portions of the kidney as usual, after the urine was free of phenol red, were

unsuccessful in inducing storage—that is, no phenol red can be identified in sections of such material.

Discussion. It is clear from the experiments summarized that 1, in a normal rabbit injected with small quantities of phenol red, this substance cannot be identified in the glomerular space even though it is present in the blood plasma in sufficient quantities to be visible; at the same time it is easily recognizable in the cells of the proximal convolution; 2, this condition is not deviated from in any essential *a*, when the concentration in the blood is increased by increasing the dosage administered; *b*, when urine formation is slowed down by dehydration and a concentrated urine is formed, and *c*, when urine formation ceases with the reduction of the blood pressure below the osmotic pressure of the blood colloids.

The method of identifying phenol red is exceedingly sensitive, but there must be a certain small minimal quantity which is not visible in thin sections. Therefore it is impossible to say with accuracy that no phenol red passes through the glomerulus. The evidence presented shows, however, that if any phenol red is eliminated by the glomerulus, the quantity so excreted is negligible compared with that eliminated by the cells of the proximal convolution. This may be correlated with the finding of Grollman (1925) that only about 5 per cent of phenol red in rabbit's blood serum is unbound and filterable.

The data presented are in general agreement with the work published by Marshall and his co-workers, but were obtained through a more direct approach.

SUMMARY AND CONCLUSION

A more direct method is utilized in localizing phenol red in the kidney of rabbits injected intravenously with small doses of phenol red. In the kidney of normal rabbits so treated, phenol red is visible in the plasma of the blood vessels and the glomeruli, and in the cytoplasm of the cells of the proximal convolution, but not in the glomerular spaces. When the blood pressure is reduced below the osmotic pressure of the blood, the results are the same. Phenol red is therefore eliminated chiefly, if not entirely, by the proximal convoluted tubule.

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OBSERVATIONS ON EPINEPHRINE OXIDATION AND STABILIZATION

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Wiltshire (1931) has reported that epinephrine, in the presence of certain tissues, is stabilized and that the stabilization is due to amino acids. The effect of amino acids on the time required for oxidation of epinephrine to physiological inactivity was studied in a specially devised apparatus in which an intestinal segment was shielded by a glass tube from contact with an epinephrine or an epinephrine-amino acid solution, so that exposure of the muscle to the solution could be effected at varying times after the epinephrine had been added. The activity of the amino acids, relative to the time requisite to the abolition of physiological activity, was reported in the following order: dl-phenylalanine, d-alanine, dl-alanine, l-tyrosine, dl-aspartic acid, l-leucine, d-arginine, dl-β-alanine, glycine, dl-cystine, sarcosine, amino-benzoic acid. It was concluded that epinephrine probably combines in some way with amino acids and is prevented from oxidation; the compound formed dissociates slowly and the epinephrine liberated is oxidized subsequently; the time of oxidation of epinephrine is therefore enormously prolonged in the presence of amino acids.

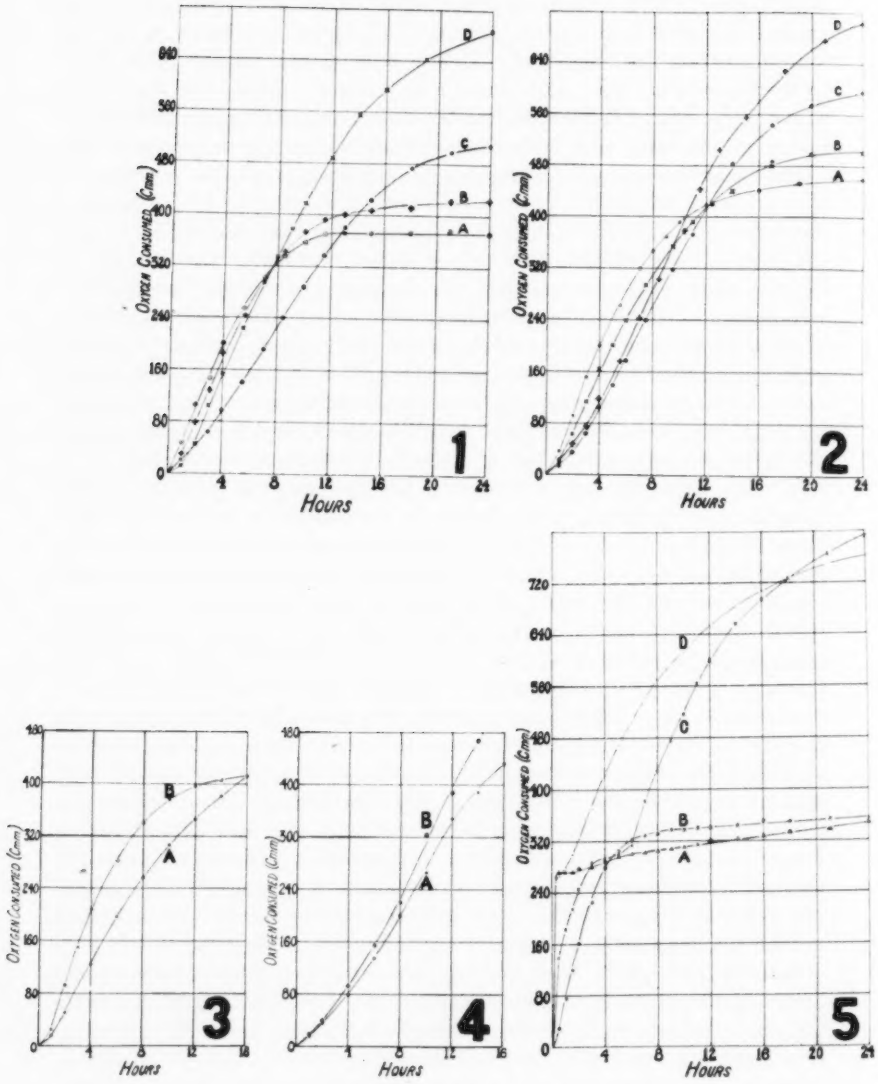
Were such a combination to occur, it seems probable that the rate of oxygen consumption of epinephrine would be more rapid than that of an epinephrine-amino acid system. Barcroft respirometers were employed to test this hypothesis.

1. RESPIROMETER EXPERIMENTS. METHODS. All experiments were performed at 38° using a M/5 mixture of KH_2PO_4 and Na_2HPO_4 at pH 7.36 (measured by the hydrogen electrode at 25°). d-Alanine (Hoffmann-LaRoche) in M/5 phosphate buffer was adjusted to pH 7.36 and the amino-nitrogen content checked by the Van Slyke method. The ratios of molecular proportions employed, i.e., d-alanine:l-epinephrine, are recorded as 500×, 100×, etc. Thus, 500× signifies 215.0 mgm. d-alanine (2.5 millimols) to 0.916 mgm. of l-epinephrine (1/200 millimol). Various preparations of natural epinephrine employed in many preliminary experiments gave unsatisfactory results; the synthetic l-epinephrine bitartrate (Farbwerke vorm. Meister Lucius u. Brüning) used in the reported experiments, was entirely satisfactory.

At physiological pH and temperature epinephrine rapidly undergoes

autoxidation, which renders equilibration of the respirometers in the usual manner impossible; in order to obtain total oxygen consumption the following method was employed. Heavy filter paper, washed with dilute hydrochloric acid, then with water, and carefully dried, was cut into a square (9 sq. cm.), folded into a smaller square (1 sq. cm.) and suspended by means of platinum wire from a glass hook within the manometer cup. Using a microburette with a very fine tip, the filter paper was impregnated with a freshly prepared l-epinephrine bitartrate solution (0.14 cc.) containing 0.916 mgm. (1/200 millimol) of epinephrine base in distilled water. Phosphate buffer solution (2.86 cc.) or alanine and buffer was then placed in the bottom of the reaction vessels. In each instance the compensator vessels contained 3.0 cc. of phosphate buffer only. When desired, carbon dioxide was absorbed on a small pledget of cotton placed inside the ground glass joints of the respirometers and moistened with 6 per cent NaOH (0.1 cc.). The instruments were shaken with the taps open until equilibration was complete, the taps closed and the filter paper tipped from the hook into the buffer solution. With this procedure, the epinephrine solution, being acid in reaction, did not oxidize until dumped into the alkaline buffer solution, and modified the hydrogen ion concentration of the latter less than 0.03 of a pH unit. That the filter paper did not influence either the rate of oxidation or the total oxygen uptake was shown from a comparison of curves obtained with and without the use of paper.

EXPERIMENTAL RESULTS. *a. Effect of d-alanine on the rate of oxidation of l-epinephrine.* Figure 1 represents several experiments carried on for 24 hours. Curve *A* shows the oxygen uptake of epinephrine, without carbon dioxide absorption, which in comparison with curve *B*, with carbon dioxide absorption, demonstrates that a considerable quantity of carbon dioxide is produced late in the sequence of reactions concerned with the oxidation of epinephrine under the conditions of the experiment. The total oxygen consumption of epinephrine after 24 hours in these experiments lies between 7 and 8 atoms of oxygen per molecule of epinephrine. In later experiments, conducted for longer periods, oxygen consumptions in excess of 8 or 9 atoms per molecule of epinephrine have been obtained; probably further oxygen uptake would be encountered at a very slow rate if the experiments were even more prolonged. It is evident that a much greater oxygen uptake occurs in the presence of 500 molecular proportions (500×) of d-alanine, and that carbon dioxide production begins from the start of the oxidation (fig. 1; curve *C*, carbon dioxide not absorbed; curve *D*, carbon dioxide absorbed). The observation that during the first 8 hours of oxygen consumption the rate with alanine present is nearly identical with the rate of epinephrine alone, indicates that stabilization of epinephrine by amino acids is not due to a combination of these substances, which slowly dissociates, as Wiltshire concluded.



Figs. 1–5

b. Oxidative deamination of d-alanine. Ammonia determinations (Folin, 1932) on the alanine-epinephrine solutions, in comparison with epinephrine solutions, after the 24 hours' oxidation period was completed, showed that considerable quantities of ammonia were produced. The consumption of oxygen and the production of carbon dioxide and ammonia indicate an oxidative deamination of the alanine brought about by the epinephrine, since the amino acid itself does not decompose under these conditions. Happold and Raper (1922) have demonstrated that ortho-dihydroxy compounds which are capable of forming ortho-quinones bring about the oxidative deamination of amino acids; it is known that the ortho-quinone from epinephrine is particularly active in this respect (Edlbacher and Kraus, 1928; Blix, 1929; Barrenscheen and Danzer, 1933). Edlbacher and Kraus found that glycine, with epinephrine as the "catalyst," liberates equimolecular proportions of carbon dioxide and ammonia, resulting in the decomposition of the glycine to the extent of 45 per cent. With other amino acids they were unable to obtain comparable results, with the exception of cystine which liberated a small quantity of ammonia. Blix, in a study of the kinetics of epinephrine as an "oxydationskatalysator," found that simultaneously with the production of carbon dioxide and ammonia, glycine gives rise to formic acid and alanine to acetaldehyde. Barrenscheen and Danzer report the identification of glyoxylic acid as an intermediate product of the action of epinephrine on glycine.

c. Effect of varying the ratio of d-alanine to l-epinephrine. Figure 2 presents curves of the oxygen uptake of epinephrine in the presence of varying amounts of d-alanine, and shows that although the initial rates of oxygen consumption did not greatly differ, the total oxygen absorbed increased in proportion to the alanine-epinephrine ratio (curve *A*, epinephrine; *B*, epinephrine plus alanine (10×); *C*, plus alanine (100×); D, plus alanine (500×). With alanine (10×) the oxygen consumption ceased within 24 hours; with greater proportions of alanine (100×, 500×) oxygen consumption continued for a longer period. It is evident that mass action affects the reaction, i.e., the greater the amount of alanine in proportion to epinephrine, the more prolonged is the oxygen consumption.

d. Effect of heavy metals on epinephrine and epinephrine-alanine mixtures. Certain minor variations observed in the experiments, which were conducted under conditions as nearly alike as possible, as well as unexplained discrepancies in the observations of Wiltshire, might partially be due to the presence of small amounts of heavy metals. Fe^{++} and Fe^{+++}, in minute quantities, produce a color with epinephrine and related catechol derivatives, which is probably due, in part, to catalysis of their oxidation by atmospheric oxygen. Warburg and others have shown the value of cyanide as an inhibitor of heavy metal catalysis in many systems. In experiments in which no attempt was made to remove the exceedingly small quantities

of heavy metals usually present in ordinary reagents, the addition of cyanide to M/500 effected no change in the rate or total oxygen uptake of epinephrine; with alanine (500×) present, the cyanide appeared to accelerate slightly the oxygen consumption.

Figure 3 shows the accelerating effect of the addition of Fe^{++} (10γ), as $FeSO_4$, on the oxidation of epinephrine (curve *A*, epinephrine alone; curve *B*, epinephrine plus iron). The course of the oxidation, following the initial acceleration, is apparently different from that obtained in the absence of iron, as evidenced by the marked decrease in the rate of the latter portion of curve *B* in comparison with curve *A*. Figure 4 shows that the addition of Fe^{++} (10γ) to epinephrine, in the presence of alanine (curve *A*, epinephrine-alanine (500×); curve *B*, iron-epinephrine-alanine (500×)) results in a slightly decreased initial, and a markedly decreased subsequent, rate of oxidative deamination of the amino acid. The addition of cyanide appears to decrease the inhibition produced by the added iron, but does not remove it.

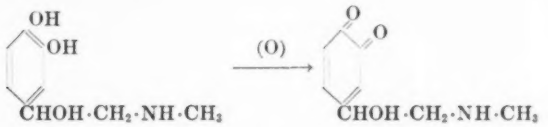
e. Effect of cysteine and glutathione on the oxidation of l-epinephrine. The investigation of the action of such tissue constituents as cysteine and glutathione, both containing a sulfhydril group and having a high reducing potential, was indicated, since their capacity as hydrogen donors might enable them to stabilize epinephrine more effectively than amino acids. Consequently the action of 10 molecular equivalents (10×) of cysteine on the oxygen consumption of the usual quantity of epinephrine was studied. In this experiment carbon dioxide absorption was not carried out. However, the important features may be seen in figure 5. Curve *B* represents the epinephrine control. The extremely rapid uptake of nearly 280 cmm. of oxygen by the cysteine (curve *A*) is theoretical for the conversion of the amount of cysteine employed (1/20 millimol) to cystine, while the slow subsequent consumption of oxygen probably indicates the conversion of the formed cystine to cysteic acid. In curve *D*, representing the oxygen consumption of the epinephrine-cysteine mixture, there is an extremely rapid conversion of the cysteine to cystine, followed by the oxidation of the epinephrine with simultaneous oxidative deamination of the cystine. In order to make the above observation more definite, cyanide was added to M/500 (curve *C*), inhibiting the catalysis of cysteine oxidation by extremely small quantities of heavy metals, and resulting in a much slower absorption of oxygen during the conversion of the cysteine to cystine, following which there is a definite lag, followed by the oxidation of the epinephrine together with the oxidative deamination of the cystine. It may be noted that the addition of cyanide resulted in a more rapid oxidative deamination of the cystine, again suggesting that heavy metals retard the rate of oxidative deamination of amino acids by epinephrine.

The observation that no red color appeared in the mixture until sufficient

oxygen had been consumed to convert the cysteine to cystine, afforded further proof that little or no irreversible oxidation of epinephrine occurred while any cysteine was present, the reduction of the quinone of epinephrine being sufficiently rapid to prevent the epinephrine from destruction.

When glutathione was used in place of cysteine in an otherwise identical experiment, exactly similar results were obtained. The rapid oxidation of the reduced glutathione to oxidized glutathione was followed by a less rapid oxygen consumption due to the oxidation of the epinephrine with simultaneous oxidative deamination of the oxidized glutathione.

Discussion. Investigators, particularly Ball and Clark (1931) and Ball and Chen (1933), agree that epinephrine, like catechol, when oxidized is at first converted to an ortho-quinone:



These authors have shown that the decomposition of the quinone thus formed is extremely rapid, occurring at a much greater rate than ortho-benzoquinone formed from catechol, and increasing rapidly with increase in pH. The irreversible decomposition of the oxidant, i.e., the primary oxidation product, of epinephrine occurs with such great ease and rapidity that it is impossible to form an oxidation-reduction system. Apparently, following the oxidation of epinephrine to the quinone-form, the quinone tends to become reduced, i.e., it is an oxidizing agent, and removes hydrogen from another portion of its own or from adjacent molecules. The steady destruction of the primary quinone in this manner results in the process going to completion with the loss of all physiological activity.

The more rapid consumption of oxygen following the addition of a trace of iron to epinephrine suggests that in the conversion of the epinephrine to the quinone, as pictured above, the hydrogen lost unites with two atoms of oxygen to form hydrogen peroxide, rather than with one to form water. Iron catalyzes oxidation by hydrogen peroxide, as has been shown in many systems, and thus increases the activity of the formed hydrogen peroxide in oxidizing epinephrine or partially oxidized epinephrine and results in a more rapid consumption of oxygen. The inhibiting effect of iron on the later stages of the epinephrine oxidation and in the oxidative deamination of alanine by epinephrine complicates such an hypothesis and remains to be further investigated.

In the presence of amino acid the quinone of epinephrine, formed as described above, has the opportunity of oxidizing, i.e., removing hydrogen from amino acid as well as epinephrine molecules; the reduction of a larger

proportion of the quinone of epinephrine to epinephrine is favored, and the disappearance of physiological activity is delayed. The hindering effect of the amino acid is doubtless modified by other factors such as temperature, hydrogen ion concentration, certain ions (other than hydrogen) and the ratio of alanine to epinephrine. The oxidative deamination of the amino acid which occurs is evidence in favor of this explanation.

Preliminary results indicate that not only iron but also substances such as methylene blue increase the oxidation rate of epinephrine, the methylene blue presumably serving in this case as a transporter of hydrogen between epinephrine and molecular oxygen.

The results with cysteine and with glutathione are such as would be expected with sulfhydril substances, or with hexuronic acid, as suggested by Szent-Györgyi (1928) and reiterated by Heard and Raper (1933). Since glutathione in the reduced form exists in considerable quantity in blood, suprarenal, liver and other tissues, and hexuronic (ascorbic) acid is also widely distributed throughout the body in tissues known to have a high reducing potential, it is difficult to picture the destruction of epinephrine through the direct agency of molecular oxygen in such tissues. An explanation is thus afforded for the pharmacological effects obtained from the subcutaneous injection of epinephrine at points distant from the sites of action.

Considering the potent action of such substances as glutathione and ascorbic acid as reducing agents it is difficult to believe that the rôle which amino acids play in the stabilization of epinephrine can be of great physiological significance. However, Wiltshire claimed that the total oxidation time of epinephrine was increased form 15 to 60 times with the varying proportions of d-alanine which she employed. Such an effect did not seem evident from the oxygen consumption curves obtained in these experiments, in which the rate of oxygen uptake of epinephrine differed little from the rate of oxygen absorption of epinephrine plus alanine. Consequently, it was necessary to determine whether the rate of decrease in physiological activity paralleled the rate of oxygen consumption.

2. PHYSIOLOGICAL EXPERIMENTS. *a. Effect of d-alanine (500×) on the rate of disappearance of physiological activity of l-epinephrine (1/200 millimol in 3 cc.).* The conditions of the experiments were identical with those of the previous respirometer experiments using alanine (500×) and epinephrine, and control experiments were carried out simultaneously in which the oxygen consumption was measured. Small quantities of solution were withdrawn from the reaction vessels of the respirometers at intervals, diluted and tested on rabbit's intestine contracting in a known volume of a Ringer solution, the pH of which was about 7.5. The addition of the phosphate buffer medium in high dilution to the intestine contracting in Ringer solution did not markedly affect the tonus or contractility;

any effect noted was always in the nature of an augmentation of contractility rather than an inhibition. The concentration of epinephrine in these experiments, containing 1/200 millimol in 3 cc., was approximately 1:3300.

After agitation in the reaction vessels of the Barcroft respirometers had proceeded for 6 hours tests were made. It was found that the epinephrine concentration in the alanine-epinephrine mixture was approximately twice that in the solution containing the epinephrine alone.

In figure 6 are shown the results of testing the physiological activity of the solutions after 15 hours had elapsed. Dilutions of fresh standard l-epinephrine bitartrate were compared with dilutions of the oxidizing epineph-

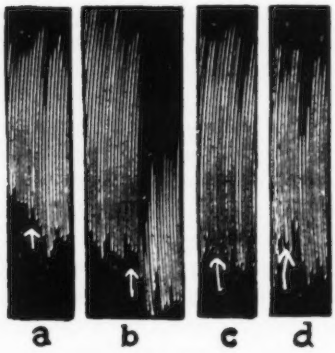


Fig. 6

a. Epinephrine – 15 hours. Diluted to 1:8 million
b. Epinephrine + alanine (500x) – 15 hours. Diluted to 1:8 million
c. Epinephrine + alanine (500x) – 15 hours. Diluted to 1:40 million
d. Epinephrine Standard – 1:250 million

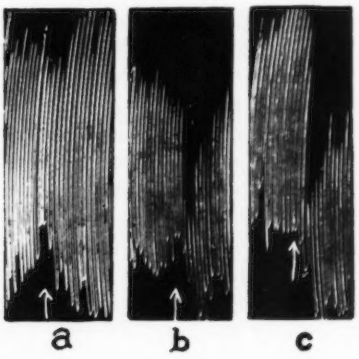


Fig. 7

a. Epinephrine – 18 hours. Diluted to 1:1.6 million
b. Epinephrine + alanine (500x) – 18 hours. Diluted to 1:6.4 million
c. Epinephrine Standard – 1:64 million

rine solutions based on the initial concentration of epinephrine, permitting the calculation of the percentage remaining undecomposed. On this basis figure 6 *a* in comparison with figure 6 *d* indicates that only 3 per cent of the epinephrine (in the absence of alanine) remained unoxidized, while figure 6 *c* in comparison with figure 6 *d* indicates that approximately 16 per cent of the epinephrine in the epinephrine-alanine mixture remained unoxidized.

Figure 7 shows the results of the tests made at the end of 18 hours of oxidation. In figure 7 *a* a dilution of 1:1.6 million of the epinephrine produced no inhibition, but rather an augmentation of contractility which is typical of the effect of a considerable increase in the phosphate content of

the Ringer solution in which the intestine is contracting. Figure 7 *b*, in comparison with figure 7 *c*, indicates that approximately 10 per cent of the epinephrine, in the presence of alanine, remained unoxidized.

Six hours later, i.e., after a total of 24 hours of oxidation, no trace of inhibition was obtained from a dilution of 1:8 million of the epinephrine in the alanine-epinephrine solution. This was the greatest concentration possible with the amount of solution that remained in the reaction vessel. That considerably more than 97 per cent of the epinephrine had been oxidized is likely when it is considered that a definite inhibition (fig. 6 *a*) was obtained with a dilution of 1:8 million and with an intestinal segment of approximately the same sensitivity, from a solution in which only about 3 per cent of the epinephrine remained unoxidized. While the final traces (3 per cent or less) of the epinephrine may have disappeared more slowly the alanine did not greatly affect the rate of disappearance of the major portion (97 per cent or more) of the epinephrine.

The rate of decrease in physiological activity was found to closely parallel the rate of oxygen consumption and the marked flattening of the oxygen consumption curves of epinephrine at 15 hours coincides with the observation that at this point less than 3 per cent of the epinephrine remained unoxidized. That a considerable amount of oxygen is absorbed after 15 hours is probably explained by the observation that epinephrine eventually takes up from 8 to 9 atoms of oxygen per molecule while it doubtless loses sympathomimetic activity following the conversion to the primary quinone.

A few experiments were performed in a further attempt to elucidate the mechanism of the remarkable effects of amino acids on the oxidation time of epinephrine, as reported by Wiltshire.

b. Observations on Wiltshire's findings. A twenty-fold increase in the time of oxidation of epinephrine to physiological inactivity was obtained in an experiment with d-alanine when Wiltshire's technique was employed, using a modification of her Ringer solution, containing M/1300 Na_2HPO_4, in place of 0.01 per cent $NaHCO_3$, to prevent pH changes as a result of aeration. Further study of Wiltshire's findings with other amino acids was not carried out, since the essential observation was thus confirmed.

The work of Blix (1929) indicates that the rate of oxidation of epinephrine varies inversely as the hydrogen ion concentration. It is suggested that the great variations in some of Wiltshire's results with d-alanine (summarized in table 1), are due, at least partially, to variations in pH, since the pH of a Ringer solution such as she used ($NaHCO_3$ = 0.01 per cent) was found to vary with the CO_2 content of the distilled water used in its preparation and increased upon aeration due to loss of CO_2; its low buffer capacity made further pH changes likely, unless substances added to it were adjusted with care. Wiltshire states that the Parke-Davis adrenaline hydrochloride solution used was adjusted to pH 7.4, suggesting that the

Ringer solution had a similar pH; no mention is made concerning the reaction of the amino acid solutions added or of the effect of aeration.

Experiments were carried out to determine whehter a greater stabilization occurred with greater dilutions of epinephrine than were used in the previously described experiments, since Wiltshire's results were obtained with concentrations of epinephrine between 1:10 million and 1:70 million.

c. Effect of d-alanine (500×) on the rate of disappearance of physiological activity of l-epinephrine (1:100,000). Experimental details in the following experiments were identical with those previously described, in which the decrease in physiological activity of epinephrine solutions in the respirometers was followed physiologically, except that the solutions were too dilute to permit controls in which oxygen consumption could be followed and M/50 phosphate buffer was employed to avoid the phosphate augmenta-

TABLE 1

ALANINE CONCENTRATION	EPINEPHRINE CONCENTRATION	MOLECULAR RATIO	INCREASE IN OXIDATION TIME
(a) M/5000	1:15,000,000	549:1	15 times
(b) M/5000	1:10,000,000	366:1	47 times
(c) 0.0035 per cent (M/2500)	1:50,000,000	3,660:1	60 times

TABLE 2

EPINEPHRINE CONCENTRATION	TIME REQUIRED FOR DESTRUCTION OF PHYSIOLOGICAL ACTIVITY		INCREASE IN OXIDATION TIME
	In absence of d-alanine	In presence of d-alanine (500 ×)	
	hours	*hours*	
1:3300	18	24	1.3 times
1:100,000	11–12	42–50	(Av.) 3.8 times

tion of intestinal contractility which otherwise would have been encountered with these more dilute solutions, containing an initial concentration of 1:100,000 epinephrine. In table 2 the results of these experiments are summarized in comparison with the results using 1:3300 epinephrine.

It was observed that 60 per cent of the epinephrine in the presence of alanine was destroyed in the time required to destroy over 97 per cent of the epinephrine without amino acid. The observation that alanine (500×) is more effective in regard to the total oxidation time with 1:100,000 than with 1:3300 epinephrine, was not extended by experiments with greater dilutions, since their activity disappeared too rapidly to permit accurate study using such technique. However, this meagre evidence suggests that the effect of amino acids in increasing the time required to destroy the physiological activity of epinephrine increases as the epinephrine concentration decreases, the alanine-epinephrine ratio remaining constant.

Further study was not conducted since it appeared that the complete stabilization of epinephrine by substances such as glutathione and ascorbic acid rendered the effects of amino acids of little physiological importance and suggested that further work to elucidate their manner of action was unjustified at the present time.

GENERAL DISCUSSION. The rate and course of the oxidation of epinephrine, or of the oxidation of amino acids induced by epinephrine, is influenced by many factors. For example, the amount of glass surface exposed to contact with the solutions in the respirometer vessels appears to affect the rate of the reaction. Iron, as well as hydrogen ions, affect both the rate and course of the reactions involving the oxidation of epinephrine and there is evidence that other ions may have an influence. Barker, Eastland and Evers (1932) present evidence which indicates a catalytic action of calcium on epinephrine oxidation. Kisch (1933) reports that cations such as Ba, Sr, Mg and Ca, particularly the latter, markedly increase the rate of oxidative deamination of glycine by certain quinones. These observations suggest that calcium may have played a part in the results reported by Wiltshire, since the Ringer solution which she used contained $CaCl_2$ (0.024 per cent). Barker, Eastland and Evers also showed that NaCl affects the persulfate oxidation of epinephrine, and Schild (1933) (in a paper appearing since the completion of this study) shows that copper definitely catalyzes the rate of development of color in the Ewins colorimetric test for epinephrine (persulfate oxidation). Schild points out that Baker and Marrian (1927) found that heavy metals influence the results obtained with the method of Folin, Cannon and Denis (1913); the latter method necessitates the oxidation of epinephrine though the reduction of phosphotungstic acid serves as a source of color. All these observations, together with the observations regarding iron, indicate the sensitivity of epinephrine oxidation to certain ions and the ease with which results may be modified.

The total oxygen consumption of epinephrine obtained, under the conditions described, does not offer a direct clue as to the nature of the oxidation product or products. It is difficult to formulate a theory to account for the utilization, at blood pH and temperature, of 8 or 9 atoms of atmospheric oxygen by a compound such as epinephrine ($C_9H_{13}O_3N$). The only hypothesis which at the present time seems worthy of consideration is that following the conversion of epinephrine to the quinone, molecular rearrangement to form an indole derivative takes place, succeeded by complex polymerization and extensive oxidation. Qualitative tests indicate that indole substances are present in a solution of oxidizing epinephrine. The red color produced when tyrosine is oxidized by tyrosinase has been shown by Raper and co-workers to be due to the quinone of an indole derivative, lying intermediate between tyrosine and melanin. The extreme instability

of the quinone of epinephrine suggests that the red color of partially oxidized epinephrine is due to an oxidation product of the primary quinone, which is probably an indole derivative. However, the course of the oxidation of epinephrine by molecular oxygen (8 to 9 atoms of oxygen consumed) differs from the oxidation by tyrosinase (5 atoms), so that the nature of the reaction beyond the primary quinone is uncertain.

The destruction of epinephrine which occurs in the animal organism is probably due to some mechanism, perhaps enzymic in nature, which is capable of bringing about the oxidation of epinephrine despite the reducing potential of the tissues. The epinephrine appears to function as a hydrogen-carrier between glutathione (and presumably other reducing substances of tissues) and molecular oxygen, but substances with an oxidation-reduction potential negative to epinephrine are present in most tissues in sufficient concentration to prevent the disappearance of epinephrine due to direct oxidation by molecular oxygen.

SUMMARY

1. A study of the consumption of oxygen by epinephrine at physiological pH and temperature has shown that the presence of sulfhydril compounds, such as glutathione and cysteine, completely prevents the irreversible oxidation of epinephrine. The presence of glutathione, ascorbic acid and other reducing substances in tissues adequately accounts for the pharmacological effects obtained from the subcutaneous injection of epinephrine at points distant from the sites of action.

2. No evidence was found to support the hypothesis (of Wiltshire) that epinephrine combines with amino acids and is thus prevented from oxidation. The rôle of amino acids in lengthening the time of oxidation of epinephrine to physiological inactivity is discussed.

3. The rate of decrease in physiological activity of oxidizing epinephrine closely parallels the rate of oxygen consumption.

4. The oxidation of epinephrine (under the experimental conditions) results in the consumption of 8 to 9 atoms of oxygen per molecule of epinephrine and the production of carbon dioxide during the later stages of the reaction.

5. Minute quantities of iron accelerate the oxidation rate of epinephrine during the primary portion of the oxidation and markedly decrease the rate during the latter portion.

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THE CHANGES IN HEMOGLOBIN CONCENTRATION OF BLOOD OF GROWING RATS

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I. Seventy years ago H. Welcker (1863; *cf.* Drastich, 1928) made the interesting observation that equal volumes of erythrocytes of different mammals have nearly the same color intensity measured with the blood-spot scale. Bürker (1922) related the Hb-content of a single red blood corpuscle to its surface and arrived at the conclusion that the Hb-concentration per unit surface of erythrocyte is constant for many mammals, with a numerical value of 32×10^{-14}. The work of Eisbrich (1924) supports Bürker's conclusion, although finding that the Hb-value per square μ of surface is about 31.7×10^{-14}g Hb.

Ponder (1924) objected to the method used by Bürker to calculate the surface area of erythrocytes. Bürker and Eisbrich measured the diameters of erythrocytes in blood films and calculated the surface by the formula

$$O = 2\left(\frac{d}{2}\right)^2$$

where O is the surface of a red blood cell and d the mean diameter of a series of measured red blood cells. Ponder's objection is that this formula does not take into account the third dimension (as it disregards the thickness of the erythrocyte), and that a red blood cell is not a coin-shaped body but is biconcave. His other objection is to the accuracy of measuring such small objects as erythrocytes with the micrometer. Ponder came to the conclusion that although the relation between Hb-content and area described by Bürker is not appreciably affected when the surfaces are calculated by a better formula, the limits of the accuracy of measurement render it impossible to decide whether there is a straight line relationship between Hb-content and surface or between Hb-content and volume of erythrocytes.

Recently Drastich (1928) took up the question; as a result of his own determinations he states that a very useful formulation is obtained by relating the Hb-content to the total volume of the red blood cells according to the formula

$$\frac{\text{Hb per 100 cc.} \times 100}{\text{volume of erythrocytes}} = \textit{constant}$$

He examined 36 individuals belonging to 11 different species and found a constant of 31.7 ±2.2 to 2.3, while the variations in Bürker's constant may be as great as 74 *per cent.*

II. The blood of the growing rat changes considerably during the life cycle of the animal; blood-count, Hb-content and size of the red blood cells show a fairly regular development. The blood count rises from 694,000 per cu. mm. in early fetal rats (Kindred and Correy, 1930, 1931) to about 10 millions in adult animals (Jolly, 1909; Chisholm, 1911, *et al*). The cell size decreases regularly with age, while the Hb-concentration increases except during the suckling period, where it declines sharply (Jolly, 1909; Chisholm, 1911). Blood samples from rats of widely different ages are like blood samples of different species. It is of some interest to find out whether any of the relationships between Hb-concentration and other independent measurements will hold during the life cycle of the rat.

The animals employed in these experiments were from an inbred strain of hooded rats. The blood was obtained in all cases by heart-puncture. It was divided into three portions. The first sample, to which a small measured amount of heparin was added, was centrifuged at about 3000 revolutions. The second sample was diluted with normal saline in blood-counting pipettes and counted with Neubauer's improved blood-counting chamber. (It is advisable to fill the pipettes up to the 5 mark for very young animals and up to the 3 mark for adults, because adults have such a high count that the work is difficult unless the blood is highly diluted.) The third sample was converted into Met-Hb and the Hb-content determined with Klett's colorimeter; by this method the error due to the micrometric method of measuring the volumes of erythrocytes is avoided. Bürker (1924) showed that the colorimetric method of Hb measurement is at least as accurate as the spectrophotometric method. The results of 12 determinations, covering the extra-uterine life span of this strain of rats, is shown in table 1. The values of the Hb-concentration increase regularly throughout life, except at the suckling period, when there is a sudden drop. The best explanation for this drop in Hb-concentration during the suckling period is given by Abderhalden (1902), who found that milk contains very little iron; although the newly born animal has a higher supply of iron in its liver than the adult the store is gradually depleted, which leads to a slight anemia during the suckling period.[1] The blood-count rises steadily during development (*cf.* Hill and Hill, 1913; Abderhalden, 1902; Addison and Richter, 1923; Jolly, 1906, 1909; Chisholm, 1911, *et al.*). I found no indications in the literature that measurements of the volumes of erythrocytes in blood samples from rats at different ages have been carried out.

[1] Ash of newborn rat and dog contains 6 times as much Fe as milk, and Fe in pup's liver is 5 to 9 times greater at birth than in the adult.

In very young animals I found it necessary to use 0.5 cc. for determinations because the animals have so little blood.

In the next-to-the-last column of table 1 there are shown the values of the Hb-content per unit volume of single erythrocytes, as suggested by Ponder. The last column contains the values of Drastich's constant for various ages. These two columns show that neither relationship furnishes a satisfactory constant. If 28.8 is taken as the mean value of the constant calculated by Drastich's method the variation amounts to ± 5.7 or about 20 *per cent.* It is interesting to note that the effects of depleting the store of iron in the animal during the suckling period seem to last for nearly 3 weeks after weaning, since the *per cent* content of Hb (expressed in the last column) does not return to the high level it had at birth until after the

TABLE 1

*Data on rat blood**

AGE IN DAYS	SEX	Hb IN GRAMS PER 100 CC.	ERYTHROCYTES IN MILLIONS PER CU. MM.	HEMATOCRIT READING	VOL. OF E μ^3	$\frac{\text{HbE}}{\text{Vol. of E}}$	DRASTICH'S CONSTANT
3	?	8.2	2.7	28.5	105	29×10^{-14}	28.87
7	?	7.8	2.8	30	107	25×10^{-14}	26.00
15	Female	8.6	3.6	31.5	88	27×10^{-14}	27.3
18	Male	8.5	3.8	33	89	26×10^{-14}	25.7
25	Male	8.8	4.2	35	83	25×10^{-14}	25.1
26	Female	9.2	4.5	35.8	79	26×10^{-14}	25.7
42	Female	10.9	5.0	40.9	81	25×10^{-14}	26.6
65	Male	12.5	5.2	41	78	31×10^{-14}	31.2
150	Female	12.8	7.4	42	57	29×10^{-14}	30.5
240	Male	14.5	7.7	42	54	35×10^{-14}	34.5
335	Male	14.4	7.9	44	55	33×10^{-14}	32.5
400	Female	14.7	8.1	45	55	33×10^{-14}	32.6

* One animal was used for each age group.

6th week of life. If one considers the effects of the suckling period upon the composition of the blood as a sort of abnormal condition—in the sense that not enough Fe is furnished in the food—the values of Drastich's constant vary but little. Some of the variations are doubtlessly due to individual differences in the composition of the blood.

SUMMARY

In examining the hemoglobin concentration of rats of various ages to determine whether the so called "constant of Drastich" is applicable during the life cycle of a single species, it was found that the Hb-concentration per unit volume of erythrocytes suffers a slight decline during the suckling period. Using Drastich's constant as an indicator it is seen that recovery

from the effects of depletion of the iron store during lactation is not complete until over 6 weeks after birth.

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THE EFFECT OF SLEEP ON HUMAN BASAL METABOLISM, WITH PARTICULAR REFERENCE TO SOUTH INDIAN WOMEN

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The average basal metabolism of South Indian women has been shown to be very low, −17 per cent compared with the Harris-Benedict and Aub-DuBois standards (Mason and Benedict, 1931). A possible explanation of this low metabolic rate is an unusual degree of relaxation. Is the Oriental, in the state of repose required for basal metabolic measurements, already in a condition of relaxation as profound as the average westerner achieves only during sleep? If so, it would be impossible for the Oriental's metabolic rate to be lowered appreciably during sleep, and the waking figures could with justice be compared only with those of westerners measured in sleep, a comparison that would considerably reduce the difference between Oriental and Occidental groups.

For satisfactory experiments on the effect of sleep on metabolism we believe the following conditions are essential: 1. A type of apparatus that allows the subject to go to sleep easily and does not leak when he is wholly relaxed. Mouthpieces are not satisfactory and face masks are questionable. A chamber may be used, but in this case the periods must be not less than 30 minutes long. 2. Strictly comparable conditions of waking and sleeping with regard to posture, digestion, and time of day or night. Experiments in which the sleeping metabolism is measured at night and the waking metabolism in the morning do not present comparable conditions nor do experiments with measurements of sleep and waking made on different days. 3. Subjects thoroughly familiar with the routine of metabolism measurements. Even a trace of apprehension or discomfort increases the metabolism above the basal and invalidates the waking measurements. 4. At least duplicate experiments, preferably more, on every subject. These conditions were all observed in the experiments reported in this paper (except that with one subject no duplicate experiment was made).

Apparatus. The oxygen consumption was measured with a spirometer type of apparatus with a blower. The accuracy of the apparatus had been established by alcohol control experiments both in the Nutrition Labora-

tory and in Madras. The subject's head was enclosed in a helmet (Benedict, 1928, 1930, 1933). On the hottest days a wet cloth was laid over the metal parts of the helmet, and the rapid evaporation under the ceiling fan prevented a rise of temperature. The temperature of the system was nearly always slightly below the room temperature.

Subjects. Seven normal South Indian women, resident students in the Women's Christian College and two western women, also living in Madras, served as subjects. The Indian women were, with one exception, selected from individuals in the lower half of the low metabolic range already found among South Indians (Mason and Benedict, 1931), on the assumption that if a change in metabolism during sleep were found in this group, at least as great a change would be found with all South Indians. The physical characteristics of these women are shown in table 1.

Procedure. The experiments (except three of those on G. E. S., which were made between 9 and 11 p.m. with the subject in the fasting condition) were made in the morning before the academic work of the day began. The subjects came to the laboratory in the post-absorptive condition (and always in the inter-menstrual period) at about 6:30 a.m. and lay down at once for a half-hour rest period. The difficulties of studying the metabolism during natural sleep in the daytime are accentuated in a hot climate where doors and windows must be kept open to allow the fullest possible circulation of air, but in spite of disturbances the subjects succeeded surprisingly well in sleeping. Each experiment consisted of several consecutive 10-minute periods, with only a minute or two between required for filling the spirometer and adjusting the kymograph drum. Tests for leaks were made in every period. Usually one or two waking periods were secured first, then sleep for as long as time permitted. The subject was then wakened and urged to stay awake for from one to three more periods. The order of waking and sleeping varied in different experiments, and there were many periods in which the subjects were partly awake and partly asleep. These mixed periods have been omitted in computing the results.

Length of rest period. Radsma (1931) in Batavia found that a half-hour rest period was frequently not long enough for his native subjects to reach a state of repose and probably not long enough for his European subjects. He therefore has adopted the routine of requiring one hour's preliminary rest for all subjects measured in the tropics. In our own earlier series in Madras (1931) there was no consistent trend in metabolism during the course of three to four consecutive 10-minute periods following a half hour of rest. The last measurement of a series was higher than the first as frequently as lower. In our sleep series, in which waking periods immediately following the half-hour rest period and others at the end of 1½ hours were secured, the last measurement was at the same level as the first in spite of the intervening period of sleep. In general it has been our ex-

perience that a half-hour rest period is enough in the tropics, but that occasionally there may be a subject who requires a longer period. This requirement is easily detected within three 10-minute periods. If there is a distinct downward trend in the waking metabolism within this time, the experiment should be prolonged until two or three successive periods agree and the first one or two high values should be discarded.

DISCUSSION OF RESULTS. *Basal metabolism.* The metabolism of each of our subjects in the awake periods, that is, under strictly basal conditions, has been averaged for all the different days of measurement and recorded in table 1. Of the seven Indian women, four had previously been studied in the experiments reported by us in 1931 and served again as subjects in

TABLE 1

Physical characteristics and waking basal metabolism

RACE AND SUBJECT	AGE	BODY WEIGHT (WITHOUT CLOTHES)	HEIGHT	SITTING HEIGHT (MEASURED)	OXYGEN CONSUMED PER MIN.	HEAT PRODUCTION PER 24 HOURS		DEVIATION FROM NUTRITION LABORATORY STANDARD
						Total	Per sq. m.	
	yrs.	*kgm.*	*cm.*	*cm.*	*cc.*	*cal.*	*cal.*	*per cent*
Tamils:								
M. K. J.	21	42.0	155	81.5	142	987	726	−20.9
K. M. C.	20	40.7	156	79.5	145	1007	740	−19.6
C. J.	22	42.1	149	73.5	139	966	732	−21.7
Malayalis:								
M. G. K.	20	45.1	159	80.0	142	987	690	−21.4
A. O.	22	45.1	162	80.0	136	945	652	−26.6
A. P. J.	19	48.2	149	79.0	159	1105	789	−11.6
Telugu:								
M. K.	20	37.7	146	76.5	139	966	773	−22.9
American:								
G. E. S.	23	53.8	167	88.5	172	1195	747	−12.9
English:								
A. V. B.	42	67.0	170	89.5	209	1452	816	+2.7

this special study after intervals of from 6 months to 2 years. All consistently showed the same low levels in this second series of measurements. The basal metabolism of the three new Indian subjects was within the range previously indicated for South Indian women, and the data as a whole confirm our earlier evidence that the metabolism of South Indian women is very low. On the average, the heat production of these seven Indians was 20.7 per cent below the Harris-Benedict standard.

Effect of sleep. The results of our experiments on the effect of sleep are summarized in table 2, the values in each line representing averages for all the measurements obtained on one day with the subject either awake or asleep (from one to four periods, usually at least two, in each state).

TABLE 2

Summary of experiments on metabolism during sleep—(average values)

SUBJECT AND DATE	RESPIRATION RATE			PULSE RATE			OXYGEN CONSUMED PER MINUTE		
	Awake	Asleep	Change in sleep	Awake	Asleep	Change in sleep	Awake	Asleep	Change in sleep
			per cent			*per cent*			*per cent*
Indians:									
M. K. J.:									
Oct. 11, 1930	16	16	±0.0	60	60	±0.0	151	131	−13.2
Oct. 17, 1930	10	19	+90.0	57	53	−7.0	144	127	−11.8
Oct. 21, 1930	14	16	+14.3	58	52	−10.3	141	132	−6.4
Oct. 25, 1930	12	18	+50.0	54	50	−7.4	133	117	−12.0
Average	13	17	+38.6	57	54	−6.2	142	127	−10.9
K. M. C.:									
Oct. 31, 1930	27	25	−7.4	64	61	−4.7	148	131	−11.5
Nov. 11, 1930	23	21	−8.7	59	56	−5.1	141	128	−9.2
Nov. 15, 1930	27	22	−18.5	65	60	−7.7	145	130	−10.3
Average	26	23	−11.5	63	59	−5.8	145	130	−10.3
C. J.:									
Aug. 20, 1930	21	18	−14.3	85	79	−7.1	137	126	−8.0
Oct. 8, 1930	19	16	−15.8	70	72	+2.9	140	125	−10.7
Average	20	17	−15.1	78	76	−2.1	139	126	−9.4
M. G. K.:									
Oct. 18, 1930	19	17	−10.5	64	60	−6.3	140	130	−7.1
Oct. 22, 1930	18	16	−11.1	84	63	−25.0	143	132	−7.7
Oct. 24, 1930	18	17	−5.6	63	58	−7.9	148	130	−12.2
Nov. 12, 1930	17	16	−5.9	65	57	−12.3	147	130	−11.6
July 23, 1931	18	18	±0.0	59	57	−3.4	132	112	−15.2
Average	18	17	−6.6	67	59	−11.0	142	127	−10.8
A. O.:									
Aug. 17, 1932	21	15	−28.6	68	56	−17.6	141	128	−9.2
Sept. 3, 1932	18	14	−22.2	60	53	−11.7	131	123	−6.1
Oct. 22, 1932	18	16	−11.1	66	56	−15.2	135	123	−8.9
Average	19	15	−20.6	65	55	−14.8	136	125	−8.1
A. P. J.:									
Aug. 9, 1930	26	19	−26.9	70	60	−14.3	159	144	−9.4
M. K.:									
Oct. 16, 1930	16	15	−6.3	67	67	±0.0	142	120	−15.5
Nov. 6, 1930	20	17	−15.0	69	67	−2.9	138	130	−5.8
Nov. 14, 1930	20	15	−25.0	68	66	−2.9	136	126	−7.4
Average	19	16	−15.4	68	67	−1.9	139	125	−9.6
Grand Average	20	18	−8.2	67	61	−8.0	143	129	−9.8

TABLE 2—*Concluded*

SUBJECT AND DATE	RESPIRATION RATE			PULSE RATE			OXYGEN CONSUMED PER MINUTE		
	Awake	Asleep	Change in sleep	Awake	Asleep	Change in sleep	Awake	Asleep	Change in sleep
			per cent			*per cent*			*per cent*
Westerners:									
G. E. S.:									
July 16, 1932.....	13	12	−7.7	59	59	±0.0	177	160	−9.6
July 22, 1932.....	13	14	+7.7	61	60	−1.6	169	159	−5.9
Nov. 29, 1932.....	14	13	−7.1	62	55	−11.3	168	158	−6.0
Dec. 8, 1932	14	13	−7.1	58	52	−10.3	176	160	−9.1
Jan. 20, 1933.....	14	13	−7.1	55	51	−7.3	168	161	−4.1
Average.....	14	13	−4.3	59	55	−6.1	172	160	−6.9
A. V. B.:									
Sept. 9, 1931	18	18	±0.0	68	65	−4.4	210	196	−6.7
Nov. 20, 1931.....	18	20	+11.1	65	63	−3.1	210	194	−7.6
Nov. 21, 1931.....	19	19	±0.0	67	62	−7.5	207	192	−7.2
Average.....	18	19	+3.7	67	63	−5.0	209	194	−7.2

It was not always easy to determine whether or not a subject was asleep. The common method of asking the subject to respond to a signal is not feasible in short-time experiments where such an arrangement might pull back to consciousness a subject just in the act of going to sleep and thus defeat the experiment.

A. Respiration. The most satisfactory gauge of dozing and sleep was the graphic record of respiration, which nearly always changed immediately as consciousness faded. The direction of change varied in different individuals as regards both regularity and rate, but each individual behaved consistently in successive trials. Our data support the conclusion that the most constant of the possible changes in respiration that may occur in sleep is in the direction of increased regularity, and that the decrease in rate is almost as constant.

B. Pulse rate. The fall in pulse rate that occurred in all subjects on going to sleep was nearly as useful an indication of the waking or sleeping state as the respiration record, but whereas the latter was continuous, the pulse rates were counted only at approximately 3-minute intervals and therefore could not show the exact moment when the change occurred.

C. Oxygen consumption. With all the Indian subjects the oxygen consumption decreased from a low waking level, already commented upon in discussion of table 1, to a still lower level. The decrease varied from 5.8 to 15.5 per cent, being less on days when the sleep was less sound or when the subjects found it difficult to keep awake. The average decrease in

metabolism during sleep was 9.8 per cent. These results indicate clearly that the low waking basal metabolism of South Indian women is not due to an extreme degree of muscular relaxation. Our experience with metabolism measurements and our observations of the life of Indians lead us to believe that the average Indian is able to drop to sleep more readily in a variety of conditions than is the average westerner, but we do not find this ability to be due to a greater degree of relaxation while awake.

In the eight experiments on the two western women, made under the same conditions as the experiments on the Indians, there was also a decrease in oxygen consumption during sleep. Comparison of our two groups of subjects suggests that the percentage change in sleep in Indians is at least as great as in westerners. An analysis of previously published experiments on adult westerners (experiments made under a variety of conditions that make them not strictly comparable with our data) shows but rarely no change and usually a decrease in metabolism during sleep. The average of all these previous experiments indicates a decrease of about 7 per cent (Loewy, 1891, 1911; Magnus-Levy, 1894; Johansson, 1897, 1898; Benedict and Carpenter, 1910; Benedict, 1915; Delcourt-Bernard and Mayer, 1925; Pickworth, 1927; Necheles, 1930; and Grollman, 1930). Although its data on sleep as such have never been assembled for publication, it is the experience of the Nutrition Laboratory that sleep lowers the metabolism about 10 per cent. In a typical experiment (the only one thus far published—Benedict, 1927, 1928) in which the conditions were most similar to ours, the exceedingly well-trained subject, Miss W., showed a fall in metabolism of 11.7 per cent during sleep in the daytime. It seems probable, therefore, that whatever the exact extent of the change in sleep in adult Occidental peoples, it is not greater than the change we have found in Indian women.

With regard to Orientals the only published account of the effect of sleep that we have found is that of Necheles (1930, 1932). His measurements on Chinese and other Orientals are hardly satisfactory for a comparison of waking and sleeping metabolism levels, because they did not meet the conditions outlined at the start of this paper as essential for such a study. For instance a comparison of the metabolism awake on one day with the metabolism asleep on another day is not justified, as our experiments on M. K. J. and M. G. K. in table 2 show, nor is the use of the drug "Somnifen" to induce sleep, as may be judged by the extremely varying effects of the drug reported in Necheles' experiments. Finally measurements during the night's sleep are not comparable with those made after waking in the morning. We believe that the variability in the degree of change in metabolism during sleep as reported by different investigators is largely ascribable to the fact that the measurements were made under conditions that did not strictly meet the requirements essential in a study of

the factor of sleep, and that, when measured in *successive experimental periods of sound sleep and periods in which the subject is motionless and relaxed but really awake*, all individuals, regardless of race, will probably show within a reasonable range of variation a decrease in metabolism during sleep of approximately 10 per cent.

SUMMARY

Seven South Indian women students, whose waking basal metabolism averaged 20.7 per cent below the Harris-Benedict standards, were studied to determine whether the low basal level previously established for South Indian women was a result of a relaxation while awake greater than that of westerners. The measurements were made with a closed-circuit helmet respiration apparatus of the spirometer type. Short-period experiments on 21 days showed a consistent decrease in oxygen consumption during sleep averaging 9.8 per cent, varying from 5.8 to 15.5 per cent with depth of sleep. This change is of the same magnitude as has been indicated for westerners. Two western women were measured on 8 days under the same conditions, and in corresponding degrees of sleep showed similar changes. These data confirm our earlier evidence that South Indian women have a very low basal metabolism when awake and show that the state of relaxation is not a causal factor in this low metabolism.

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DIRECT ELECTRICAL STIMULATION OF DENERVATED AUTONOMIC EFFECTORS

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Several studies of the results of direct electrical stimulation of smooth muscle have been published (see Ritchie, 1932, for references). The muscles, however, were innervated. It was therefore impossible to exclude an excitation through the nerves. Smooth muscle does not degenerate on denervation, but still responds quite efficiently to chemical stimuli (adrenin, acetylcholine, etc.). The present study deals with the responses of denervated autonomic effectors to electrical stimuli.

Effectors having a double antagonistic innervation are undesirable for these observations, because the electrical stimulus might excite the two antagonistic processes and thus possible responses could be decreased or masked. We therefore selected structures known to possess a single autonomic innervation: the adrenal medulla, the nictitating membrane, the pilomotors and the non-pregnant uterus of the cat.

METHOD. Cats were used, under dial anesthesia. The denervation of the effectors was performed aseptically from 7 to 22 days before the observations. Removal of the superior cervical sympathetic ganglion denervates the nictitating membrane. Section of the splanchnics and removal of the upper abdominal sympathetic chain destroy the nerve supply of the adrenal medulla. Extirpation of the abdominal chain denervates some hairs. The denervation of the uterus is difficult, since the sympathetic relay occurs in the diffuse hypogastric plexus (Langley and Anderson, 1896). In the three experiments on the uterus, to be reported below, the lower abdominal chains and the inferior mesenteric ganglia were removed; a uterine horn was ligated and cut at its vaginal end, leaving the ovarian artery as the sole supply of blood; the arterial border of the uterine horn was painted with concentrated phenol.

In all cases denervation was unilateral and the innervated side was used as a control. The absence of degeneration of the denervated effector was tested by recording the responses to chemical stimuli: adrenin for the nictitating membrane and the uterus, and acetylcholine for the adrenal gland (Feldberg, Minz and Tsudzimura, 1933). The pilomotors were not tested thus, for their response to adrenin is negligible.

The electrical stimuli used were induction shocks from a Harvard inductorium with 8 volts on the primary circuit and also rectangular shocks (direct current) from a "multivibrator" (see Rosenblueth, 1932b). The application of the electrodes will be described below.

Cocaine was commonly used to enhance the responses of the nictitating membrane (Rosenblueth and Rioch, 1933). Curare was injected to eliminate perturbing contractions of striped muscle.

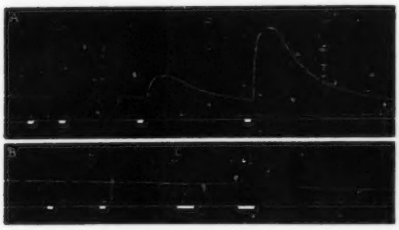

Fig. 1

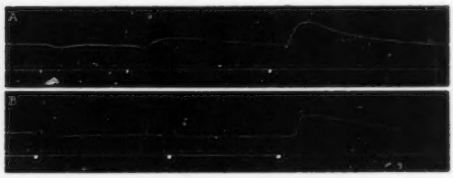

Fig. 2

Fig. 1A. Contractions of the nictitating membrane elicited by direct electrical stimulation of the right (innervated) adrenal. Dial, cocaine, curare. Inductorium; coil distances, 9, 8, 7.5 and 7 cm. In this and other figures the time signal records intervals of 30 seconds. In all records of the nictitating membrane the magnification was the same.

B. Absence of contraction when the left (denervated) adrenal is stimulated after removal of the right gland. Coil distances, 7 and 5 cm.

C. Left (denervated) adrenal stimulated by direct current from the multivibrator. Duration of the current: 200 σ. Intervals between stimuli: 200 σ. Potentials applied: 25 and 40 volts.

Fig. 2. Same preparation as in figure 1. Responses of the nictitating membrane to successive injections of acetylcholine.

A. Right adrenal ligated. Left (denervated) adrenal intact. Doses in conventional units: 1, 3, 6.

B. Both adrenals ligated. Doses (in the same units as in A): 6, 10, 40.

Isotonic responses of the nictitating membrane and the uterus were recorded by means of levers. The movements of the hairs were observed directly.

RESULTS. I. *Adrenal medulla.* The nictitating membrane, sensitized by cocaine, was used as an indicator of adrenal secretion (Rosenblueth and Cannon, 1932). Wire electrodes were placed tangentially on the surface of the gland at a distance of about 6 mm., on either side of the lumbo-adrenal vein, thus insuring a relatively large contact. This contact was maintained throughout the observations by fixing the electrodes with a clamp, to avoid possible mechanical stimulation.

The innervated gland secretes readily when stimulated by means of induction shocks at tetanizing frequency, as shown in figure 1A. The

same type of stimulation is ineffective on the denervated gland (fig. 1B). The direct current is likewise ineffective (fig. 1C). The length, intensity and frequency of the shocks from the vibrator can be increased till visible damage of the gland occurs, but no demonstrable adrenin is secreted.

That the denervated gland is capable of secretion is shown in figure 2A (the innervated gland had previously been removed). Increasing doses of injected acetylcholine elicit increasing responses of the nictitating membrane. These responses are due to secreted adrenin, and not to the acetylcholine itself, for they disappear after removal of the gland (fig. 2B). The direct response of the nictitating membrane to acetylcholine requires larger doses (fig. 2B).

Fig. 3. Direct electrical stimulation of the nictitating membrane. Dial, cocaine, curare. Adrenals ligated.

A. Stimulation of the innervated nictitating membrane with induction shocks. The first 7 signals mark tetanizing frequency; the coil distances were 9, 8, 7, 6, 5, 4 and 2 cm. The last record registers the effects of single shocks, as marked by the signal; coil distance, 0.

B. Stimulation of the denervated nictitating membrane with induction shocks. The first 7 signals mark tetanizing frequency; the coil distances were 7, 6, 5, 4, 2, 4 and 5 cm. The last record registers the absence of effects of single shocks as marked by the signal; coil distance, 0.

II. *Nictitating membrane.* Needle electrodes were used. One was placed under the skin in the nose, and the other was inserted lengthwise into the cartilage of the nictitating membrane at its free border, immediately below the attachment of the serrefine connected to the recording lever.

The results of stimulating an innervated membrane with induction shocks at tetanizing frequency and variable intensity are shown in figure 3A. Single induction shocks of adequate intensity are also effective, particularly after cocaine (fig. 3A). Longer stimuli from the multivibrator likewise elicit responses, which vary within a wide range according to the

intensity and the frequency. The responses are in all cases similar to those obtained from stimulation of the nerve supply. The latency is relatively short (about 1 second). The subsidence of the contraction begins shortly after cessation of the stimulus.

Electrical stimulation of the denervated nictitating membrane, on the other hand, evokes quite different results. Single induction shocks are ineffective (fig. 3B). The results of repetitive stimulation, either by induction shocks at tetanizing frequency (fig. 3B), or by means of the multivibrator, are irregular and inconsistent. The latency of the responses is usually long (from 3 to 6 seconds, sometimes longer). In all but one of the 8 cases studied the threshold for these responses was higher than that of the innervated side.

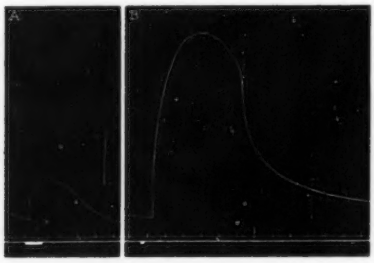

Fig. 4A. Highest contraction elicited by direct electrical stimulation of a denervated nictitating membrane.

B. Contraction of the same membrane caused by 1 cc. adrenalin, 1:200,000, at the end of the experiment.

There is no correlation between the end of the stimulus and the beginning of relaxation. The contraction may begin to subside during stimulation or, not infrequently, may persist long after stimulation ceases. In some cases there was a further rise after cessation of the stimulus. No precise correlation was found between the intensity and frequency of stimulation and the height or duration of the ensuing contractions.

The responses of the denervated nictitating membrane vary largely from one preparation to another. In 3 animals induction shocks produced almost no contraction. Figure 3B represents a typical case of a responsive preparation.

Whether induction shocks or longer rectangular waves were used to stimulate, in no case was there any response comparable in height to those which may be obtained by adrenin. Figure 4A illustrates the greatest response obtained from electrical stimulation of a sensitive denervated membrane. This response was much smaller than the contraction elicited by 1 cc. adrenalin, 1:200,000, at the end of the experiment (fig. 4B).

The long latency and great variability of the responses of the denervated membrane to electrical stimulation suggested the possibility of reflex humoral effects—i.e., adrenin or sympathin. The adrenals were therefore removed and the liver nerves cut in 5 cases. The responses persisted with the same general characteristics (fig. 3B). The most salient effect of the operations was a smoother base-line—i.e., decreased rhythmic activity. A spontaneous rhythm persisted, however, in some instances under these conditions.

III. *Pilomotors.* The hairs were clipped with scissors and two needles inserted under the skin. Stimulation with induction shocks at tetanizing frequency produces readily an erection of the innervated hairs, especially marked at the regions of insertion of the electrodes. This erection is quite similar to that obtained from stimulation of the nerve supply.

Fig. 5

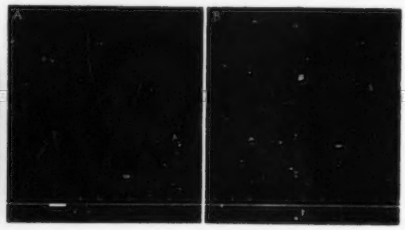

Fig. 6

Fig. 5A. Direct stimulation of an innervated non-pregnant uterus. Inductorium; coil distance, 4 cm.

B. Response of the same uterus to 0.1 cc. adrenalin, 1:200,000.

Fig. 6A. Contraction of a denervated non-pregnant uterus on direct electrical stimulation. Inductorium; coil distance, 5 cm.

B. Relaxation of the same uterus on the injection of 0.1 cc. adrenalin, 1:200,000.

The same stimuli applied to the denervated regions failed to elicit any visible response, even when the intensity of the shocks applied was higher than that sufficient to give marked responses on the innervated side.

IV. *Non-pregnant uterus.* Here again needle electrodes were used, inserted at the two extremities of the horn. The lower one, connected to the end through which the vessels entered the muscle, was firmly held by a clamp. The upper one was movable and was placed immediately below the thread leading to the recording lever.

Electrical stimulation of the innervated side invariably yielded relaxation, more marked with stronger or more frequent shocks, like that obtained from stimulation of the hypogastric nerves, or from adrenin (fig. 5). Very long rectangular waves (up to 1 second) from the vibrator likewise elicited relaxation at all intensities. Even stimuli sufficient to produce visible injury near the electrodes failed to evoke contraction.

In the three "denervated" (see method) preparations studied, one relaxed in response to the electrical stimulus, another was apparently inexcitable by the current and the last one definitely contracted (fig. 6A). All three were inhibited by adrenalin (fig. 6B). It is difficult to determine histologically the complete absence of nerves. The operative procedure used is likely to damage the muscle and it gives rise to extensive adhesions with the neighboring structures. For these reasons we did not carry on the observations further.

The results obtained permit us, however, to draw some inferences. Since the inhibitory response to the electric stimulus disappeared in two cases, we assume that the preparation in which this response persisted was not thoroughly denervated. Since, in the other two, adrenin was still effective, we assume that denervation does not interfere with the receptive mechanism, just as it does not interfere with the contractile receptive mechanism of the nictitating membrane. Other inferences will be taken up below.

Discussion. It is generally taken for granted that effectors, muscle particularly, are directly excitable by the electric current. In view of the results reported here this assumption is unwarranted. The denervated adrenal gland is electrically inexcitable (fig. 1) although the secretory effector system is present and susceptible of activation by means of acetylcholine (fig. 2). The innervated non-pregnant uterus is electrically inexcitable, merely inhibitable (fig. 5)—according to current concepts of excitation as opposed to inhibition.

The responses of the denervated nictitating membrane are strikingly different, both quantitatively and qualitatively, from those given by the innervated structure (fig. 3). The contractile effector mechanism is unimpaired by denervation, for the denervated muscle is more responsive to adrenin and its maximal contractions are as great as those obtained from the innervated side (figs. 3A and 4B).

Single induction shocks, which elicit appreciable twitches from the innervated nictitating membrane, fail to stimulate the denervated organ (fig. 3). This might suggest an increased chronaxie brought about by denervation. Lapicque (1926) has described a similar phenomenon in denervated striped muscle, and has stressed the point that the chronaxie increases as the muscle degenerates. Smooth muscle, however, does not degenerate on denervation. Furthermore, if we were dealing with increased chronaxie, we should expect that adequately long and intense stimuli would evoke responses similar to those from the innervated side. The differences between the denervated and the innervated sides would then be merely quantitative, and not qualitative, but this is not the case. The anomalous shape of the responses, the lack of correlation between cessation of the stimuli and beginning of relaxation, the variability of the responses, are all inexplicable in terms of an increased chronaxie.

The smooth muscles in the pilomotors and in the nictitating membrane have many common physiological characteristics. Both receive a single excitatory nerve supply; both respond to a single volley of nerve impulses; both present very little or no spontaneous rhythmic activity. The results of direct electrical stimulation of the denervated muscles, however, are apparently different (see pp. 386–388). Possibly the difference is merely quantitative. As stated above, some denervated membranes exhibited practically no responses to the electrical stimulus.

The atypical responses of the denervated nictitating membrane to electrical stimulation and their lack of correlation with the characteristics of the stimuli (beg nning, end, intensity, duration and frequency) suggest that denervation may abolish the electrically excitable system responsible for uniformity and correlation. The stimuli might then act directly on the contractile system. Probably the cathodal contracture and Gelfan's (1930) graded contractures in striped muscle are also due to effects of the current on the contractile system (see Adrian, 1933). All these phenomena might, therefore, be similar. The evidence available is not sufficient, however, to affirm this similarity. The mechanism of the contractile system is still obscure. It is interesting, however, that two of the latest theories, Hill's (1926) and Meyer's (1929), postulate charged protein molecules. An electric current might not be without influence upon such a system. The variability of the results obtained from the nictitating membrane is in harmony with the extreme complexity of the reactions involved—thermic phenomena, polarization, etc.

If we accept the possibility of a direct action of the current on the contractile system, the results obtained on all the denervated structures may be readily explained. In terms of the schema presented by one of us (Rosenblueth, 1934) for the sequence of functionally correlated events which lead to the specific reaction of the effector, we suggest that denervation of autonomic effectors causes a degeneration of the mechanism responsible for the production of the chemical mediator, leaving the receptive and the contractile or secretory systems intact. Direct stimulation of innervated effectors evokes the usual responses because it activates the production of the mediator. This activation may occur through the nerves. The denervated adrenal gland fails to respond because the secretory system is unaffected by the current.

The results obtained on the non-pregnant uterus are covered by the interpretation suggested. Electrical stimuli evoke exclusively "inhibition" of the innervated muscle because they cause the production of the mediator, adrenin or a closely related substance, which in turn causes "inhibition" of the contractile system. It is interesting to note that adequate chemical stimuli, e.g., ergotoxine, elicit a contraction. Although the observations on the "denervated" uterus were few and not uniform, we feel that the

one case in which adrenalin produced relaxation and electrical stimulation caused contraction was especially significant. The results on the two denervated uteri can be explained as follows. Denervation removes the mediator-producing system. Then, in one case "inhibition" was abolished, and in the other the direct effects of the current on the contractile system appeared (fig. 6A).

The explanation proposed does not imply that the production of the mediator is a function of the nerves. It merely states that the system responsible for this production is trophically dependent on the nerve supply. In striped muscle the trophic dominance of the nerve extends to the contractile system itself, since the whole muscle degenerates after denervation.

"Inhibition" was written with quotation marks in a previous paragraph because the results reported in this paper and those discussed in a former publication (Cannon and Rosenblueth, 1933) require that the concept of inhibition be revised, as applied to smooth muscle. The classical concept of inhibition implies the decrease or cessation of a pre-existing activity (Bayliss, 1915). It is therefore a passive phenomenon. In smooth muscle the classical concept of the activity to be depressed is tone. Tone in turn is defined as persistent excitation (Bayliss, *loc. cit.*).

The classical concepts of inhibition and tone are, to be sure, satisfactory in some cases. Thus, in central inhibition, as pointed out by Sherrington (1929), c.i.s. has no direct effect on a motoneurone, but cancels c.e.s., the excitatory agent. Tonic activity in striped muscle is known to be merely a fusion of asynchronous incomplete tetani in the different units of the muscle in question, originated by asynchronous discharges in the nerve supply. A similar tone may be found in smooth muscle in some cases—for instance, in the nictitating membrane normally retracted, but protruding on section of the cervical sympathetic or on administration of certain anesthetics. An inhibition of this type of tonic activity is a central inhibition of the excitatory discharge.

But the classical concepts for tone and inhibition do not fit the phenomena observed in other types of smooth muscle. Smooth muscle disconnected from the central nervous system—indeed, totally denervated—is still capable of being "inhibited" (fig. 6B). Furthermore, as is well known, this type of "inhibition" can be produced by nerve impulses, which usually are associated with excitation, or by physiological products, hormones—adrenin and acetylcholine—which elicit contraction of other smooth muscles, thereby revealing their excitatory efficiency. The studies on chemical mediation (see Cannon, 1933) lead to the conclusion that both "excitatory" and "inhibitory" nerve impulses evoke the same immediate response in muscle, the liberation of a chemical mediator. The specific reaction of the effector depends on the nature of the receptive substance (Cannon and Rosenblueth, 1933). So-called peripheral inhibition of

smooth muscle appears thus as a *response*, not as a cessation of continued contraction resulting from responses to persistent excitation. This view is in accord with Howell's (1925) statement that inhibition is something more positive and characteristic than simply the quelling or blocking of an action in progress.

The quest for the hypothetical exciting agent responsible for sustained persistent contraction of smooth muscle has been fruitless. The nerves have been definitely eliminated and the hormones as well, since the state persists in excised, perfused preparations. Ritchie's (1928) suggestion, that rhythmic activity and tone may be explained if it is assumed that passive stretch is a stimulus and that the muscle then enters into a long (several seconds or minutes) refractory period, is open to serious objections. Unstretched smooth-muscle cells, e.g., isolated cells in tissue cultures, beat rhythmically (Lewis, 1920), and unstretched, excised smooth muscle may be inhibited (Langley, 1905—observations on the amnion of the chick). An unstretched non-pregnant uterus of the cat will visibly relax when its nerves are stimulated or adrenin is supplied. The melanophores of amphibians—conceived as modified smooth muscle (Spaeth, 1916)—which are unstretched, are at rest in the contracted state (Hogben, 1924), and those of *Fundulus*, when denervated, assume an intermediate stellate condition and may expand or contract under the influence of different substances (Parker, 1932). Furthermore, nobody has succeeded in demonstrating a long refractory period in smooth muscle.

The proof of the existence of tonic activity in denervated smooth muscle, i.e., of persistent excitation leading to persistent moderate contraction, requires the demonstration of the acting stimulus. Let us consider whether we can abandon this concept and still explain "inhibition." Merely to make our ideas more concrete we shall select a model of the elastic system of the muscle based on current theories. These theories, though elaborated for striped muscle, may be regarded as applicable to smooth muscle. The truth of the model will not affect the concept; other models can be dealt with in a similar manner.

Meyer's (1929) theory of muscular contraction postulates changes in pH in the muscle which give rise to changes in the charged condition of the muscle protein. He conceives the change to be from salts of inorganic cations and protein anions toward the isoelectric zwitterions. In the zwitterionic condition the molecules would become shorter and contraction would ensue. Edwin J. Cohn (personal communication) points out that changes in the charges due to variations of the pH within physiological limits are quite improbable, for the titration curve of myosin is almost horizontal between pH 5 and pH 9 (Salter, 1926; Edsall, 1930). On the other hand, Cohn suggests that the influence of ionic strength on the solubility of the protein is such as might explain the changes in the elastic

properties of the system observed. The solubility of myosin as a function of the ionic strength of the solvent is shown schematically in figure 7 (see Cohn, 1932; myosin behaves like edestin). Contraction would then be due to a decrease of the ionic strength, rendering the protein more insoluble. This view is in accord with the increase of the viscosity of striped muscle during contraction, demonstrated by Gasser and Hill (1924). The decrease in ionic strength might be due to the breakdown of trivalent phosphocreatine into divalent phosphates. These digressions indicate that the theory is plausible.

Let us now assume that stimulation of the nerves which induce relaxation produces an increase of the ionic strength of the system, while that of the nerves inducing contraction decreases the ionic strength. The muscle may be at rest (equilibrium with a given ionic strength) at different points in the curve of figure 7. According to the classical concept of "tone," equilibrium at rest would exist when the proteins of the muscles are entirely dissolved—i.e., at the point of maximal relaxation—as indicated by the dotted arrow. Tonic excitation would lower it to the full arrow, whence it could be raised by inhibition or lowered further by excitation. In order to permit either contraction or relaxation without involvement of the concept of existing "tone," we suggest that the resting position may be at or around the point marked by the full arrow.

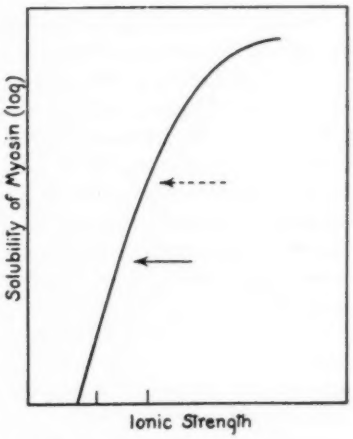

Fig. 7. Schematic representation of the solubility of myosin as a function of the ionic strength of the solution. The probable physiological range of ionic strengths is marked. For explanation see text.

There is evidence that excitatory and inhibitory effects of nerve impulses are due to the appearance of different substances (Cannon and Rosenblueth, 1933). A substance which would shift the equilibrium upwards would produce relaxation, and one which would shift it downwards contraction. Thus transient reversible changes in the elastic properties of the system would occur—responses—which can bear opposite signs. This theory is in accord with the similarity of quantitative relations found between degree of nerve stimulation (Rosenblueth, 1932b) or concentrations of adrenin (Rosenblueth, 1932a) and degree of response, both for contraction and inhibition.

In terms of the schema presented, a distinction is to be made between

muscles which are at rest in the region of the dotted arrow (e.g., mammalian striped muscle, heart, nictitating membrane and pilomotors) and those which are at rest in the region indicated by the full arrow (e.g., striped muscle in the claw of arthropods and smooth muscle with a dual nerve supply). In the former only positive variations—contractions—are elicited by the stimuli; in the latter both positive and negative variations may occur. The melanophores of amphibia (see above) might represent the other extreme of the series; resting equilibrium would lie here lower in the curve, in the fully contracted state (Hogben, *loc. cit.*), and only a negative variation—expansion—may be obtained.

The model schematized in figure 7 offers, likewise, an approach to the problem of rhythmic activity of smooth muscle. Such activity might be a series of periodic oscillations around a mean which represents equilibrium. The cause of these oscillations remains to be determined. The baffling relation existing between "tone" and rhythmic activity has been stressed by one of us (Cannon, 1911). A certain degree of shortening of the muscles in the colon and stomach is important for the appearance of rhythmic activity. As figure 7 shows, if equilibrium stands above the dotted arrow, large changes of ionic strength would be necessary to elicit rhythmic oscillations, while very minute changes will evoke such oscillations if equilibrium is in the region of the curve marked by the full arrow.

The nature of this paper is such as not to warrant further speculation on these subjects. But we would emphasize that a physicochemical approach, even if only in terms of a provisional model, is more likely to lead to further research than the indiscriminate use of vague biological terms.

In order to render more precise certain biological terms, we make the following suggestions. The use of the word tone should be restricted to the cases in which the classical concept of a persistent excitation holds, e.g., for tone in striped muscle or in the innervated blood vessels and the nictitating membrane. In this sense denervated smooth muscle does not possess tone, so far as we know. The term inhibition should be restricted to cover the instances of decrease or cessation of previous excitation, e.g., central inhibition or inhibition of the pace-maker in the heart by the vagus, inasmuch as the pace-maker is the periodically active source of the excitatory wave which conditions the beat of the heart. Denervated smooth muscle, not contracted by hormones, cannot be inhibited, according to this view, because it is not previously excited, so far as we know. Two types of responses may be obtained from the nerve impulses impinging on it, which, needless to say, are always excitatory agents: contraction and relaxation. We select this second word advisedly to substitute for "inhibition:" we we would emphasize that the mechanical changes in muscle involve changes of the dynamic equilibrium which are fundamentally of the same nature, but bear opposite signs.

SUMMARY

The responses of the denervated adrenal gland, nictitating membrane, pilomotors and non-pregnant uterus to direct electrical stimulation were studied in cats, and compared with the responses obtained from the corresponding innervated effectors.

The denervated adrenal gland (fig. 1) and pilomotors (p. 388) appear to be electrically inexcitable. The denervated nictitating membrane yields responses which are qualitatively and quantitatively different from those obtained from the innervated muscle (figs. 3 and 4). The innervated non-pregnant uterus responds exclusively by relaxation (fig. 5). This relaxation may be replaced by contraction after denervation (fig. 6).

The behavior of the denervated effectors is explained as due to the disappearance of the mediator-producing system (pp. 389–390).

The concepts of "tone" and "inhibition" of smooth muscle are discussed (pp. 391–392). In some cases the classical concepts apply (p. 391); in others it is more in accord with the evidence available to consider "inhibition" as a response of relaxation (p. 392).

A model for the contractile system of smooth muscle, based on a theory suggested by E. J. Cohn (unpublished), is presented and discussed (fig. 7 and pp. 392–394). The conclusions stated in the preceding paragraph agree with the implications of Cohn's theory and with those of other current theories of muscular contraction (pp. 393–394).

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RELATION OF INTENSITY AND DURATION OF BRIEF RETINAL STIMULATION BY LIGHT TO THE ELECTRICAL RESPONSE OF THE OPTIC CORTEX OF THE RABBIT[1]

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In beginning an analysis of the activity of the rabbit's optic cortex, Bartley and Bishop (1933a, b) in the interests of simplification and control, used direct electrical stimulation on the optic nerve rather than normal stimulation through the sense organ (eye) to elicit a cortical response. There was obviously some information that could be obtained only in this way. But on the other hand, a complete functional study of the optic pathway cannot neglect to ascertain the possible difference in cortical outcome between direct optic nerve stimulation and the normal stimulation of the nerve through the eye. The processes in the two cases differ in two respects. The first difference is that in stimulating a nerve directly, only one impulse in each fibre follows each stimulus, while in activating a sense organ (the retina) even the briefest effective stimulus presumably results in repetitive discharges into the nerve. Consequently in one case the frequency of impulses in the nerve is a direct correlate of the rate of stimulation, while in the other it is determined by the rate of discharge of the sense organ, and so cannot likewise be controlled. In the second place the way in which the particular fibres of the optic nerve are selected for activation is different in the two cases. The difference is brought about in part by the way stimulus intensity and duration function in electrical stimulation in contrast to stimulation of the retina by light. The fibres of the optic nerve like those of other nerves are of assorted sizes, and their thresholds to electrical stimulation vary in accordance with size, so that the largest of them are activated by the weakest stimuli and the smallest by the strongest. At the same time, no one fibre size is segregated to a single portion of the nerve's cross-section. As a result of these facts, no particular portion of the nerve's cross-section is selected at any time by an electrical stimulus, but simply more and more of the fibre population of each unit of cross-section is activated as either stimulus intensity or duration is increased. From the standpoint of fiber activation then, there

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are three variables, the size of fibre stimulated from only the largest at first to the smallest also as the stimulus increases in strength, the total number of fibres activated and the number of active fibres in each unit of cross-section. These parallel each other and cannot be separately controlled.

Changes in stimulus strength and duration act differently in the case of retinal stimulation. They first change the number of retinal elements active for the same reason that electricity varies the number of fibres active in the optic nerve, that is, on account of a range existing in the thresholds among the retinal-element population. Intensity and duration may also change the frequency of discharge into the optic nerve.

There is another kind of possibility with retinal stimulation; namely, that fibres of particular portions of the optic nerve cross-section can be experimentally selected as desired. This occurs when a stimulating quantity of light falls only on a portion of the retina. The satisfaction of this condition is more difficult than it might at first seem. Such a situation does not ordinarily occur and did not exist in the stimulus situations in our experiments, for there is reason to believe that even with a stimulus subtending a small visual angle and surrounded by a dark field, there is sufficient light scattered about in the eye to stimulate the whole retina as well as simply the region occupied by the retinal image. However, the more intense stimulation of one portion than another exemplifies the principle of spatial selection in the nerve cross-section which does not occur with direct electrical stimulation of the optic nerve itself. This selection of course is the sensory basis of space perception in the animal, and can conceivably be manipulated in a way to reveal further details in the functioning of the optic pathway.

But it was not the aim of the present investigation to ascertain in detail the degree of similarity between the two sets of results. We have attempted to show the effect of varying intensity and duration of the flash upon the time required for the cortex to begin to respond. This time we shall call the *implicit time* both in order to avoid the repetitive use of a cumbersome phrase such as "the time of the appearance of the response" or the "time interval between the beginning of stimulation and the response" and to avoid such terms as latent time and reaction time. The term latent time has been variously used and in a case closely similar to the present work has been employed to designate a portion of the time used by the photoreceptor mechanism to stimulate nerve. There is a variety of "reaction times" in use, so that the selection of a new term has been thought advisable.

The measurement of the implicit time is made from the beginning of stimulation to the peak of the first response potential. The peak rather than the onset of the potential was chosen on account of the fact that the

onset is not clearly defined for cortical records continually show potentials of spontaneous activity. The peak of the response is the most clearly defined characteristic and inasmuch as the response wave seems to be of rather uniform duration, the peak bears a steady relation to the onset.

The response. A typical response of the cortex to a brief retinal stimulus consists of two recognizable components, the first of which is frequently a diphasic wave though sometimes modified and the second of which, following about 30 sigmas later, is generally monophasic. They are shown in plate I. The first phase of the diphasic and the monophasic wave are of a polarity that indicates the surface of the cortex is positive at the time with respect to an indifferent point on the skull. The unanalyzed features of the first component (diphasicity and polarity) compare to those of the first component obtained with direct electrical stimulation of the optic nerve. But on the other hand the total response is less prolonged as far

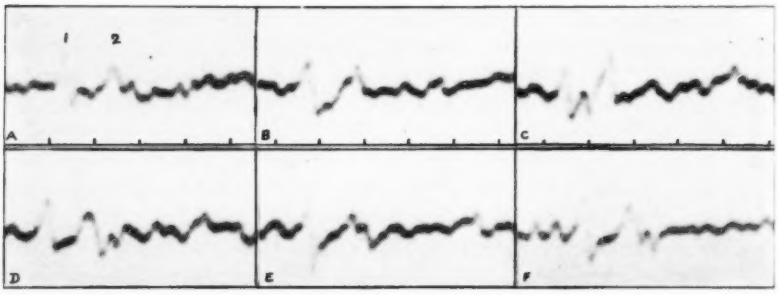

Plate I. Specimen responses to brief flashes of light. Time marks in the plate are 30 sigma units. Wave *1* in A is first component, *2* is second. Component *1* is often several times as high as *2*. Beginning of stimulation precedes peak of response by about 48 sigmas.

as at present can be determined. Direct electrical stimulation produced five components instead of two and they appeared at a rate of about 5 per second instead of about 30 to a second. In electrical stimulation, the first component was found by analysis to be a compound of two waves, a large diphasic and a small monophasic. Such an analysis was possible on account of their difference in threshold and their separation in time at low intensities as well as their separation with the application of certain drugs to the cortex (Bartley, 1933). With retinal stimulation modifications in the shape of the first component as well as the manner in which the wave reduces in size with waning stimuli would lead one to suspect the component is also compound in such cases. However, a detailed analysis will have to wait until later.

Further description will be confined to the first component except where

otherwise specified, and the component will be called the response as if no other waves followed it. This response varies in several ways, some of which are spontaneous, while others are clearly controllable.

In the first place, the time of appearance seems to vary somewhat from trial to trial. It is common for several responses to appear at a constant time, only to be followed by one or more at some different time, a little earlier or later. Part of this shifting is in connection with a change in the wave form, and both seem to result from the summing of the response with potentials in the spontaneous activity of the cortex, so that in certain individual cases the actual time of occurrence does not coincide with the time as superficially indicated. The timing of the response determined by the nature of the stimulus will occupy the greater part of the paper.

The size of the response also fails to remain constant. There is an inherent rhythm in it similar to that occurring in the cortex with electrical stimulation of the optic nerve (Bishop, 1933). With prolonged stimulation the response may vanish and fail to return till a rest is given. Stimulus characteristics themselves, however, play a part in determining the size.

METHOD. The recording was done as described previously (Bartley and Bishop, 1933a), by vacuum tube amplification and a cathode-ray oscillograph. A stimulating system which would provide for light flashes of considerable range in duration and intensity, as well as for sufficient intervals between them had to be arranged. It was necessary also that the system provide for a stimulus of considerable area.

The arrangement which met our requirements was essentially the following. Two and sometimes three revolving discs interrupted the light from a lamphouse, falling upon a small slit in a screen. The image of this slit and the animal's eye were at conjugate foci of a 3 inch biconvex lens of 8 inch focal length. The whole lens was homogeneously supplied with light and the size of the retinal image was then controlled by a series of apertures regulating the amount of lens used. The light intensity was controlled by a rheostat and ascertained by a voltmeter, a voltage-brightness curve having been previously constructed for this purpose. In this way intensities ranging from below that which would produce a recordable response up to 2400 candles per square foot were obtained.

Durations ranging from a little less than one sigma up to 37 sigmas were produced with our particular speeds and angular openings of the discs mentioned above. The high speed disc was so made that its open sector could be varied at will while the disc was in revolution. The error introduced by such a device was about 1 degree. With high speeds no appreciable time variation was made in the flash and its rise to maximum and decay were both small in proportion to the duration time.

With this variable disc, it was possible to manipulate the stimulus duration while observing the response and see even small changes in the implicit time if they occurred.

The slower discs regulated the intervals between stimuli; these ranged from one to three seconds, and except for times of actual observation or measurement, experimental stimulation was avoided. During all of the experiments the animal was kept in a darkened room.

The animal's eye was held rigid by clamps fastened directly to the eye tissues after the severing of some of the extrinsic muscles. This was found necessary, for our degree of anesthesia did not prevent eye shifting which at times interfered with our measurements.

The relation of continued stimulation to implicit time. Figure 1 shows two sets of readings taken under the same stimulus conditions. In both cases implicit time was plotted against stimulus intensity. The set represented by the series of dots was obtained by increasing the stimulus step by step, the other set was taken in the reverse order immediately afterwards.

This simple experiment is a test of whether continued stimulation has some effect upon implicit time through adaptation. If so the effect would be greater the more the trials and the more intense the stimuli. In the experiment the readings differ in keeping with the order taken. In the first set the intensities are becoming greater step by step and implicit time is lengthened more on that account at the upper end of the curve than at the lower. The second set, being taken immediately afterwards, begins at approximately the value that the first series ended with, and implicit time is still being lengthened by the same means as the second series continues, but as the intensities become less the effect making for the lengthening of implicit time becomes less so that the two sets of readings finally become identical again and the lower ends of the curves approximately coincide as do the upper ends. And so it is that continued stimulation works toward changing implicit time especially if the stimuli are intense. It is likely that this effect is slight if a number of seconds' interval is allowed between stimuli or if the stimuli are no stronger than the weaker ones that we used. In a number of the test cases the two sets of readings were virtually identical, and in cases in which a sudden change to certain previous stimulus conditions was made for a check, the new values coincided very well with the old. Since this phenomenon has not been studied in detail this brief mention will suffice. It does at least caution one to stimulate the eye as little as possible in making measurements and to remember that this potential influence enters into a series of readings in such a way that the readings should be plotted in the order that they are taken if the greatest consistency is to be obtained. For example, where implicit time is measured in connection with variations in two stimulus factors the one changing from reading to reading, and the other one changing after each complete series (see figs.), the curves follow the order of the readings.

The relation of stimulus intensity to response. When the eye receives flashes of light varying in intensity, concomitant variations in implicit time occur. The upper limit of this effect lies somewhere above 2400 candles per square foot, the most intense light used in the experiments, although at this level as evidenced by the example in figure 1, the light has reached most of its effectiveness, and stronger light no matter how intense,

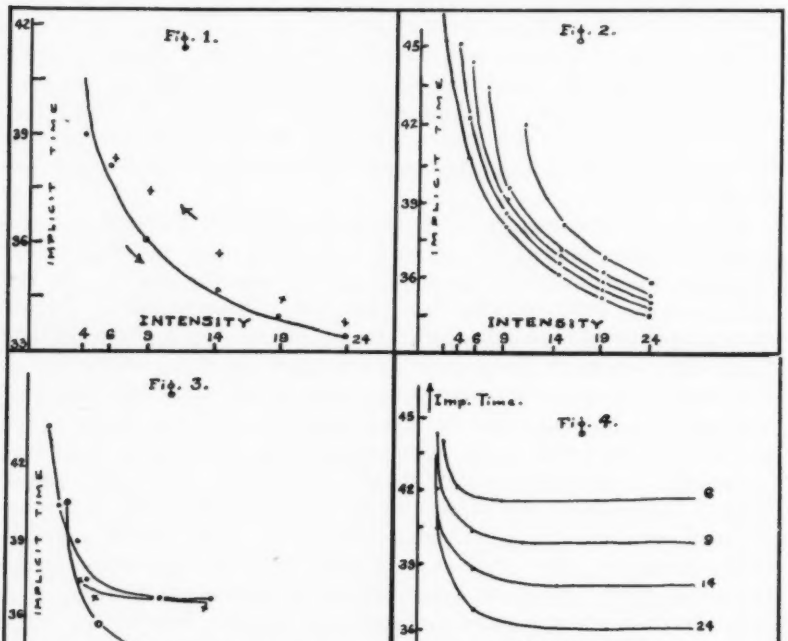

Fig. 1. Curve showing variation in implicit time with continued stimulation. Ordinate in sigmas. Abscissa in units of 100 c./ft.²

Fig. 2. Graph of a number of composite intensity curves. Same units used.

Fig. 3. Three curves showing the relationship of duration of stimulus to implicit time. Units given in sigmas.

Fig. 4. The relation of quantity of light and implicit time. Quantity in units of 1 sigma × 2400 c./ft.² (a relative measure).

could not shorten implicit time more than a very few sigmas. At the other extreme, the response usually disappears when intensities much below 150 candles per square foot are used, though in a few cases even 20 candles per square foot were effective.

The lower end of the curve in figure 1 if extended a little way would in-

dicate a theoretical implicit time of indefinite length, or in actuality no response at all, and this at an intensity level not a great deal below the lowest one that we found to give a recordable response. At least part of the difference in the value of the theoretical minimal effective stimulus and our actual minimum may be accounted for by the fact that waves of spontaneous cortical activity are continually present in the records, and would obscure the very smallest responses. These spontaneous potentials can never be eliminated except by means which would at the same time change or eradicate the very response itself.

Figure 2 is a family of intensity curves constructed from the information obtained in a number of experiments. Each curve denotes the intensity-implicit time relation for a different duration of flash. It shows that even within the range that duration (vide infra) has its effect in varying implicit time, the intensity curves still almost parallel each other.

It is apparent from the minimum intensity of light needed to provoke a recordable response, that our weakest stimulus is not the smallest one that will arouse sensation. It is simply the weakest one that will give us an electrical record considering the fact that the rest of the cortex is always active and its activity is also electrical, and the incoming messages cannot be distinguished from each other as if they were things of separate categories and could be identified by color, or other simple differentia. It is noteworthy in this connection that in human experiments at or near threshold, the external stimuli not under study must be reduced to a minimum and as nearly as possible to a uniformity if the lowest and most reliable measurements are to be obtained. In fact, in addition to this, the subject himself must put forth uncommon effort in the one direction of dealing with the experimental stimulation if these threshold measurements are to be assured. In other words, the message sent to the brain in such cases is so tiny that it can easily be ineffective or become lost, as it were, in the greater activity of the brain. In distinction to this, we are measuring in our situation the electrical aspect of nervous activity. The messages never mean anything mental to us as for the experiencing subject. We have only their size and position in time to identify them for us and consequently they must be large enough to be picked out above the other activity which is also electrical and as such does not differ from the incoming messages from the retina. We have then only the upper end of the effective stimulus scale left for our purposes, but it can be seen that we have sufficient left to tell a great deal about its relation to cortical response.

Relation of stimulus duration to implicit time. The duration of the stimulus is also a determiner of implicit time, but the variations produced by changes in it are not as great as those produced by changes in intensity. For the conditions used in our experiments, variations in duration brought about approximately half as much change in implicit time as did intensity

and no change was produced till the duration became very short. This is similar to the effect obtained by Adrian and Matthews (see their curves 1 and 3 on pp. 281, 283, 1927–28). Our figure 3 illustrates the results of three different experiments. It is apparent that the absolute values of the implicit time are not identical in the examples but it is equally true that the place at which duration begins to become materially effective in changing implicit time is about constant from case to case, when intensity

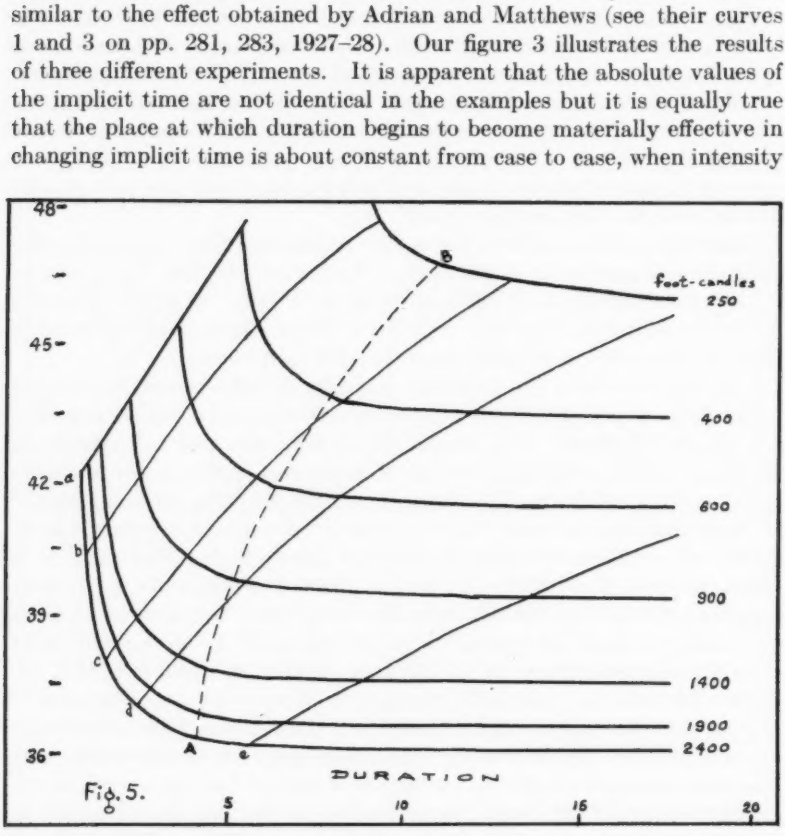

Fig. 5. A family of curves showing relation of duration, intensity and quantity to implicit time. Each numbered curve represents a single intensity with varying duration. Curves *a*, *b* and *c* denote equal quantities throughout their courses. Curve *AB* denotes critical duration.

is not altered. Figure 5 pictures a family of duration curves constructed from the data of a number of cases. The members of this family show how duration acts at several levels of stimulus intensity. It is obvious that with higher intensities the stimulus flash may be made shorter without lengthening implicit time, than with low. For example, with an intensity of 2400 candles per square foot the implicit time begins to become noticeably longer when the duration is reduced to the neighborhood of 6 sigmas while at 400 candles per square foot a corresponding lengthening in implicit time

takes place at 10 sigmas. Further similarities between the findings here and in other studies will be brought out in the discussion.

Relation of quantity to implicit time. A more simple set of relations between stimulus and implicit time may be obtained by using the product of intensity and time (quantity). Figure 4 shows this from the data of a number of experiments. The rising parts of the curves finally all come to a place of coincidence, showing that for very small quantities and low intensities the implicit time depends simply on the quantity of light received until the duration has approached the critical value for the given intensity of light. Beyond this, the implicit time soon reaches a steady value determined by intensity alone. Figure 5 contains a set of constant quantity curves, which show that this effect is certainly not true for most of the intensity values used in our experiments. Constant quantities wherein the factors of time and intensity are varied shift implicit time in some cases as much as 8 sigmas. However, to increase implicit time 8 sigmas, the stimulus duration has to be increased much more than this amount, suggesting the possibility that the stimulus begins to be effective at various places in its duration in keeping with the intensity used. The family of curves in this figure does not include a curve for the minimal effective intensity and so cannot demonstrate the simple quantity relation between stimulus and the response. That was shown by figure 4. The curves were pointed out here, however, to show that this relation does not hold for intensities and durations which are not the smallest.

DISCUSSION. The main facts relating to implicit time in this paper are similar to those describing certain other processes occurring in the chain of events from stimulus to sensation. These resemblances, which are those between implicit cortical time, and the reaction times of the retina and optic nerve, as well as the "action time" of visual sensation, will be pointed out.

The effect of duration of flash upon the reaction time of both retina and optic nerve has been studied (Adrian and Matthews, 1927, and 1927–28). It was shown that the temporal relation of the two to duration is similar, except that a constant retinal-nerve interval is interposed between the two discharges. The curves for the relation of both functions show that over a considerable range of flash durations, the reaction time remains constant, but when duration is shortened below a certain critical value, depending upon intensity used, the reaction time is lengthened. Hecht showed this same function for duration in a simpler organism. In the present study the implicit cortical time has been found to behave with reference to stimulus duration, in the same way as does each of these other processes.

Hecht analyzed photoreception into two processes, namely, the primary sensitization process (stimulus duration) and the secondary process during which the substance formed in the first period acted in some way to stimu-

late the nerve. This second period he called the latent time. He presented curves to show that the reciprocal of the latent time was a linear function of the sensitization period, and stated that the reciprocal of the latent time was directly proportional to the first period, or the quantity of light acting. If the implicit cortical time is divided likewise into two parts, the first, the stimulus duration, and a second, the remaining time, a linearity exists between the first and the reciprocal of the second as long as the critical stimulus duration is not exceeded.

Adrian and Matthews found a linear relation between quantity of light and the reciprocal of the reaction time for both retina and optic nerve. The curve for the optic nerve contained the constant retinal-nerve interval, while of course the retinal curve did not. In neither case however did a direct proportionality exist. The slope of our curve for implicit time and its relation to the graph's origin more nearly resemble those of nerve rather than those of the retina.

Furthermore, Adrian and Matthews showed that for short flashes of different intensity the reaction time depends upon the total quantity of light received by the eye, a bright flash of very short duration producing the same effect as one of less intensity and longer duration. This is true for flashes somewhat shorter than the critical duration for the particular intensity of light used. When the flashes exceed this duration, the reaction time comes down to a steady value when it is then varied by intensity alone.

They also showed that the critical duration increased as the intensity is reduced, and that the product of the two is not constant, diminishing as lower intensities are used. That is, the quantity of the light required to reduce reaction time to a steady value is smaller with low intensities than with high. The same kind of change in required quantities was found in McDougall's study (1904) of "action time" for light flashes, that is, the time required to produce maximal brightness in sensation for a given stimulus intensity. For instance, as the intensity was reduced from an arbitrary unity to 1/64 of it, the quantity necessary was reduced from 49 units to 1.5 and the action time was increased from 49 to 100 sigmas. Here again in the relations pointed out, the implicit cortical time copies the optic nerve in its temporal behavior, and in addition the behavior of a known attribute of visual sensation.

Certain curves of the graph in figure 5 demonstrate this matter very well. The curves *a*, *b*, *c*, etc., are so superimposed upon the duration curves for the several intensities that each represents an equal quantity at all points in its course. That means then that if a certain quantity of light were required to reduce implicit time to a steady value, let us say at an intensity of 2400 candles per square foot, and one wished to note the implicit times at which this steady value were reached for other intensities,

it might be expected that one should find the given point on the 2400 unit curve and follow the constant quantity curve to the points of its intersection with the curves for other intensities, thereby reaching the determinations. But this is not to be done. If any point be chosen on one duration curve to represent the critical duration and a line drawn through the homologous points on the remaining duration curves as line A and B, one will find that the line does not coincide with any imaginary equal quantity curve throughout its whole course, but rather occupies a position which shows that as intensity is reduced the quantity indicated becomes less until low intensities are reached. The curve then about parallels the equal quantity curves in its neighborhood.

The measurement of implicit time does not carry with it precise information as to what proportion of the total time is used by the eye in stimulating the nerve, and the time used for conduction and synapse action. The fact that implicit cortical time was manipulated in a way closely paralleling the reaction times of the retina and the optic nerve serves as a gross indication that most of the implicit time was consumed by the eye in getting the message started. The most direct method, that of recording optic nerve impulses themselves, has up to the present failed us. But evidence is to be taken from the direct electrical stimulation of the optic nerve, in which the shortest time elapsing between stimulation and response was 5 sigmas or less. This seems to indicate that conduction to the cortex occupies only a small fraction of the total implicit time. A second method, that of recording from the eye-ball, has given us some information. In making such readings, we alternated with cortical records by the throw of a switch in the lead-off circuit, so that conditions in the two cases would be comparable. The response from the eye was a positive wave, which for all we know was the B wave of the usual retinal response. If so, then the retinal response (A wave) had begun only a short time before. This B wave appeared 2–4 sigmas later than the cortical response, indicating that the cortical response does begin only a short time after the beginning of the optic nerve discharge.

SUMMARY

The onset of the cortical response to stimulation of the retina with light has been studied by varying the intensity and duration of the light flashes.

1. Intensity is effective over a considerable range in varying the implicit cortical time. The latter is defined as the interval between the beginnings of stimulation and response.

2. Duration of flash is also effective in changing implicit time. However, this duration must be very short. For intensities of 2400 candles per square foot, the duration must be as short as 4 or 5 sigmas to materially lengthen implicit time.

3. The manner in which intensity and duration change implicit time is comparable to the way these variables affect the reaction times of the retinal and the optic nerve discharges. Thus in time to onset of activity, the cortex resembles the behavior of the eye in its variations.

4. There is the possibility of further study of spatial factors in this method.

I am greatly indebted to Drs. G. H. Bishop and G. A. Fry for their suggestions and criticism.

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INDUCED OCULAR LUMINESCENCE AS A PROBABLE SOURCE OF ENTOPTIC VISION

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Entoptic phenomena link in a peculiar way the fields of physiology and psychology. Their ready perception demands, perhaps, a training of the sensory organ comparable to that needed in the motor apparatus for use in feats of skill. At all events, fixation of attention is usually fundamental to the perception of those sensations which we commonly regard as aberrant from the functional uses of the eye.

This paper treats of two different entoptic expressions of the retinal vascular apparatus. The first is that of moving points of light, like tiny yellowish stars, apparently representing brief spans of the retinal capillary circulation.

The second is a static form of vascular shadows familiar in the projection of so-called Purkinje figures.

Recognition of the first phenomenon may be facilitated by preliminary practise in the development of another with which it might be confused, which is very similar in visual quality but wholly different in origin.

The latter phenomenon is readily evoked in the rested eyes in the dark by their vigorous rotation in any direction.

The visual result of an effective movement is a momentary flash of discrete bright points which stipple a considerable and definite portion of the field of view, according to the direction in which the eyeballs have been rotated. The sensory apparatus invoked is apparently extremely subject to fatigue, and the bright points are devoid of any circulatory movement. The luminous points are rather definitely localized in the field of view corresponding with the direction of rotation; they are confined to its lower half on looking downward, to its upper half on looking upward. With lateral movements of the eyes there is far less perfect localization of the luminous stippling, though it preponderates on that side toward which ocular rotation is directed.

The luminous rotations fail to respond after a few repetitions of the eye movements; they probably are due to direct mechanical stimulation of optic nerve fibres. No opinion is here offered as to the loci of the nerve stimulations, though it may be noted that the distribution of the referred

sensations in the field of view is that which should be expected were the effective stimuli due to sudden *stretching* of the fibres in the main nerve trunks at the optic foramina.

This assumes that when looking down or up, fibres on the upper or under surface, respectively, of the optic nerve trunks are stimulated by sudden tension.

Having identified the star-like flashes thus demonstrated by mechanical stimulation of the optic nerves, the observer can more easily apprehend the first of the two phenomena to be described.

This consists essentially in the perception of traces of the retinal circulation as manifested in luminous corpuscular movements in the dark after sudden extinction of external illumination, as when at night an electric switch is turned off, or in the daytime when the observer moves quickly from a well-lighted into a dark environment. The quality of the punctate luminous sensation now considered is similar to that referred to above, attributed to mechanical stimulation, but its brightness is usually much less; and whereas the points of light produced by ocular movements appear fixed, those following cessation of illumination distinctly move, usually in short curvilinear paths. Efforts to produce the phenomenon may at first be fruitless, but having once clearly identified it, patient observation always restores it; fatigue apparently plays little or no part in the mechanism involved.

Most of the writer's studies were made incidental to bed-reading at any hour of the night.

An electric bulb of 40 to 60 watt power, supplied by the municipal current, shielded by the ordinary parabolic reflector, was supported by a floor standard on the left of the observer, approximately at the level of the eyes and within a distance of about two feet. Exceptionally a narrow arc of bright light from the far rim of the reflector fell upon the temporal side of the left eye, but most of the light entered the pupils after reflection from the printed page held at an easy reading distance.

Observations were made under various conditions, as after steady reading from a few minutes up to an hour or two, or reading on awaking in the small hours or in an effort to cajole sleep. At a chosen moment the light would be snapped off and the attention concentrated on the visual field. To avoid ocular movement the eyelids usually remained open, but often they were gently closed and covered with one hand; this manœuvre seemed to lend a precision and intimacy of retinal perception not wholly accounted for by lessened objective illumination. Having observed the fundus in the dark, timing events when necessary by the radial pulse, the light was turned on and brief pencil notes made of what had been seen.

Wide differences marked the results of the demonstrations; often they were negative, especially on first trial, frequently faintly positive, but not

seldom "brilliant." On turning off the light, after lapse of a second or two there might be perceived a few widely scattered faintly luminous points, obscurely moving usually in the extreme periphery of the field of view, soon to fade permanently. Repeating the experiment, without well defined alteration of conditions, subjective or objective, there might appear a shower of numerous luminous points, apparently thickest in the periphery but invading the whole field of view.

However numerous the moving stars, they gradually fade until the last is extinguished after a maximum life of 20 to 40 seconds; exceptionally, a highly brilliant point lingers awhile near the middle of the field. The impression is gained that single light points have but brief existence, to be succeeded by others of like character, as when in a passing procession each marcher reflects for an instant a fixed beam of light.

To the trained observer these phenomena are apt to intrude unexpectedly in the field of view whenever objective illumination of any sort is suddenly diminished, especially after physical exertion or bending the body, which apparently tends to interrupt the regularity of the retinal circulation, as Rood (1) pointed out.

I have never succeeded in apprehending the exact correlation between the entoptic visualization of the retinal circulation and the ensemble of physical, physiological and psychological conditions which determine it. A too glaring and extensive objective illumination, or such as induces strong after images is highly unfavorable, probably because the faintly luminous motes described are obscured by contrast. What may be called the cortical atmosphere has extraordinary determining influence.

My notebook is emphatic in contrasting the relatively brilliant demonstration of the entoptic circulation an hour or so following a stimulating social-scientific gathering as compared with the feeble results from the same experimental routine without such antecedence.

That such effects connote merely psychic relativity is shown from the same notebook which records exceptionally brilliant results in experiments made during a return to mental norm after a day of marked depression. In brief, for a successful demonstration the intensity of incident light and the duration of exposure should exceed certain minima.

Freshness, or "dark accommodation," of the retina is less important, but highly desirable. Sedulous practice assures accomplishment, suggesting analogy to musical execution. Most of my observations were made upon a retina doubtless harboring residual images.

According to Adler (2) "dark adaptation" of the eye may take more than an hour for its completion, though over 60 per cent of this sensitivity is attained in the first five minutes (p. 198). He dwells on the exquisite sensitivity to movement in the periphery of the retina as contrasted with the fovea, and this property would seem to be correlated with the usual peripheral predominance of the entoptic images now discussed.

While the arrangement for bed-reading as described, making possible immediate transition between any grade of illumination and complete darkness, admirably fulfilled most experimental requirements, some of the most satisfactory demonstrations of the thesis were spontaneous and unexpected, as when, after being busied for some minutes in a rather brightly illuminated room, one suddenly entered another which was in darkness. A habit of appropriate attention is the peculiar desideratum.

It might be supposed that illumination limited to ultra-violet or infra-red rays entails peculiar effects. Through the courtesy of a colleague, L. G. Crosby, I was enabled to make tests with the use of therapeutic apparatus, but my rather superficial observations were completely negative.

It would needlessly complicate this presentation to dwell upon the correlation between the physical dimensions of the blood corpuscles and the sensory elements of the retina (3, ii, 309; 4, ii, 807; 2, 198).

Still more difficult would be an estimate of our experience if its substantiation depended on a demonstration of its accord with the law which postulates that a sensation varies with the product of the intensity of stimulus into its duration (2, 195). Even physical measurements and enumerations of retinal structures may have to be revised; it has recently been estimated, e.g., that the number of foveal cones per square millimeter surface is actually more than eight times greater than hitherto supposed (5).

Strange indeed that optical works, so far as I know, make no mention of the phenomena here described.

Helmholtz (3, i, 218), describing the projection of shadows of retinal vascular figures by means of an external light moved back and forth, notes that at the *beginning* of an observation the vascular figure is apt to flash brightly for a moment; this is because the external light falls upon a part of the retina previously shadowed by the vessels.

This appearance, of course, is not to be confused with the phenomenon here described. As quoted in the volume of Helmholtz edited by v. Kries (ii, 310), Purkinje narrates the visual sensations that burst upon him when, after a half-hour's walk in bright sunshine he entered a relatively darkened room: "I notice . . . . innumerable extremely tiny luminous points moving amongst each other along various lines, piling up now here, now there," etc. The picture presented by Purkinje is more resplendent than is likely to be reproduced, but the conditions of its formation were essentially the same as those which have concerned us, namely, a sudden diminution in external illumination, causing a subjective impression of luminous movements attributable to the retinal circulation.

We must now inquire into the mechanism engaged in the production of our phenomenon. An explanation that immediately occurs is that the writer has depicted simply an unusual example of the familiar "positive after image" as applied to the retinal circulation. But the difficulties in

maintaining this thesis seem greater than those in the way of that now proposed.

My interpretation of the phenomenon is founded on the optical properties of the ocular media, most pronounced in, if not confined to, the lens. *Fluorescence,* which quickly disappears on withdrawal of the incident light, and *phosphorescence* which lingers notably after it (1, 62; 4, ii, 820).

We find in the autonomous luminescence of the lens an obvious basis for all the phenomena here described.

The proximity to the retina of the source of light in this case must modify essentially its effects, for it must reach areas of the retina refreshed by rest in the vascular shadows cast by external light; particularly we may assume extraordinary illumination of the peripheral zones which are so exceedingly sensitive to motion; moreover, the available energy of the radiation must be enhanced by proximity to the retinal screen. The appearance of luminous moving corpuscles replacing one another through a period of 20 seconds or more, if due to radiation from the lens as suggested, must be defined as phosphorescence.

But there is another expression of what seems to be a somewhat similar phenomenon which has been described by several observers as "streaming" visual movement. It would seem that this term is used with varied significance (3, i, 223), but always it connotes coincidence of sensation with the external illumination; therefore if mediated through optical properties of the lens it must be known as fluorescence. To the writer a "streaming" effect is concomitant with rather strong, extensive, unfocussed objective illumination, and is obscurely sensed as a centripetal tide of bright motes most obvious in the periphery of the field.

The important researches of Burge have given a meaning to the fluorescent properties of the lens which signalizes an exquisite protective adaptation of the mechanism (6). The story turns on the innate lethal properties of ultra-violet radiation for living cells, involving coagulation of their protein. The conclusion reached by Burge is, "that the fluorescent property of the lens protects its protein from precipitation by ultra-violet radiation" (6, 35).

Adler (2, 209), writes: "The degree to which a fluid or tissue absorbs light depends largely on its protein content. . . . The lens contains the largest percentage of protein than any tissue in the body. . . . lens affords considerable protection to the retina by absorbing the injurious short-waved light. . . . The thermal rays beyond the infra-red pass through all the media of the eye and are the cause of many characteristic eye diseases."

Some years ago Sewall (7) sought to point out the saving grace of adaptations by which the light-leaking structure of the side walls of the eye both enhanced its esthetic range and improved its acuity of vision.

It was supposed that the essential facts of the foregoing presentation had been securely established, when the writer removed temporarily to a wholly different environment on the seashore. Here, 45 days after the last nocturnal experiment had been noted, it was thought desirable to make a few confirmatory observations. The experimental conditions were assembled as usual, but when the observer snapped off the light not a coruscation appeared in the field of view! Many times on two successive evenings the experiment was repeated under varied conditions with but feeble positive responses. Then, almost suddenly, the corpuscular flashes began to reappear and soon attained a satisfactory brilliance.

We now consider the second entoptic phenomenon on which this paper is based. In the absence of the peculiar subjective "colored clouds" of the retina, the unlighted field of view of the "dark-adapted" eyes gives me the impression of a homogeneous, dark, velvety surface. But after disappearance of the entoptic luminous corpuscles described above, the subjective appearance of the fundus is not homogeneous but rather a patchwork in which the prevailing tone is comparable to that of a sheet of platinum under feeble illumination, a somewhat opalescent sheen. Reconsidering the data of an experiment, within about a minute of turning off the external light there becomes outlined on approximately the whole retina, but more conspicuously on its platinum-like sectors, a dark to black branching network, sometimes springing from a single trunk. Very similar in appearance is the shadow of a leafless tree cast upon the ground by rather feeble moonlight. These figures begin to appear shortly after fading of the luminous corpuscles previously described; they continue to increase in complexity, and improve in definition for many seconds and may endure for several minutes. They seem invariably to attend on the experimental procedures described regardless of the luminous corpuscles. We can only infer that these figures represent the shadows of retinal blood vessels; they even develop on rare occasions a fine capillary network surrounding a central clear area, presumably the macula.

Complexity is frequently added to this picture by the appearance of extensive smoothly outlined areas of uniform blackness. It is surmised that these opacities probably represent areas of vascular congestion.

The assumption that this configuration pictures the vascular shadows of the retina involves the responsibility of determining the source of light under which they are formed. It might fairly be argued that they are after images of the blood vessels produced by the external light which had entered the pupils. Strongly opposed to this supposition is the fact that the definition of the branching shadows on the self-illuminated screen of the eye seems nowise impaired by preceding ocular movements. Moreover, all objective demonstrations of vascular shadows require continued movement of the incident light, which at any instant discloses only vessels transected by the line of motion.

The view here presented is that the figures develop subsequent to turning off of the external light, and that they represent the shadows cast by the blood vessels on a retina faintly illuminated by the phosphorescent lens. In a darkened room the configuration may be projected on a sheet of white cardboard held in the hand and barely visible.

Finally, in keeping with the teleologic character of the adaptations which give functional value to physical accessories of the eye that would themselves seem useless or even of evil portent to vision, the writer ventures the statement that the patient observer in this field is likely to acquire the impression that subconscious stimuli originating in the retina, perhaps chiefly through the blood circulation, may be of profound importance in regulating the threshold of irritability and the lability of certain of the higher nerve centres.

To sum up: Two different entoptic phenomena, apparently hitherto undescribed, have been detailed. Both are features of the retinal circulation and their visualization is attributed to luminescence of the ocular media, notably the crystalline lens.

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REACTIONS OF THE RAT UTERUS, EXCISED AND IN SITU, TO HISTAMINE AND IN ANAPHYLAXIS

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The response of the actively or passively sensitized isolated rat uterus is one of contraction (Parker and Parker, 1924; Kelloway, 1930). Since histamine in dilutions up to 1:1,000,000 depresses the uterine activity (Guggenheim, 1912; Führer, 1913; Gunn and Gunn, 1914; Longcope, 1922; Voegtlin and Dyer, 1925) Kelloway concluded that in this species histamine was not the responsible agent in the anaphylactic contraction of the smooth muscle. However, Best and McHenry (1931) pointed out that there is some ground for the belief that very minute amounts of histamine may stimulate the rat uterus (Abel and Macht, 1919). Moreover Katze observed this reaction in some instances (see Feldberg and Schilf, 1930, p. 168–169). The rat is peculiarly tolerant to histamine shock as well as to anaphylaxis, and markedly more sensitive to both conditions after the removal of the suprarenals (Flashmann, 1925; Scott, 1928; Crivellari, 1927; Marmorsten-Gottesmann and Gottesmann, 1928; Wyman, 1929). It seemed, therefore, that a comparison of the respective reactions to histamine and in anaphylaxis on the part of the isolated and non-isolated uterus in normal rats as well as in doubly suprarenalectomized rats with and without autoplastic cortical transplants, might yield further information on the nature of this discrepancy.

METHODS. Young ($2\frac{1}{2}$ to 6 months) virgin rats from a mixed stock of colored varieties of the albino were used. Experiments were done one to two weeks after suprarenalectomy and six to twelve weeks after autoplastic transplantation of the cortex (no demonstrable medullary tissue being present). For the experiments on anaphylaxis, sensitization was induced by 3 to 5 intraperitoneal injections of 0.5 to 1.0 cc. of horse serum, and tests were made on the 9th to 14th day after the last injection (Parker and Parker, 1924).

The myograph of the excised uterus was obtained by the usual method. Ringer-Locke solution was used in preference to Tyrode's, since the latter tends to depress the normal activity (Kennedy, 1925; Heller and Holtz,

[1] This problem was suggested by Dr. Leland C. Wyman, to whom I wish to acknowledge my sincere thanks for his helpful criticism and advice.

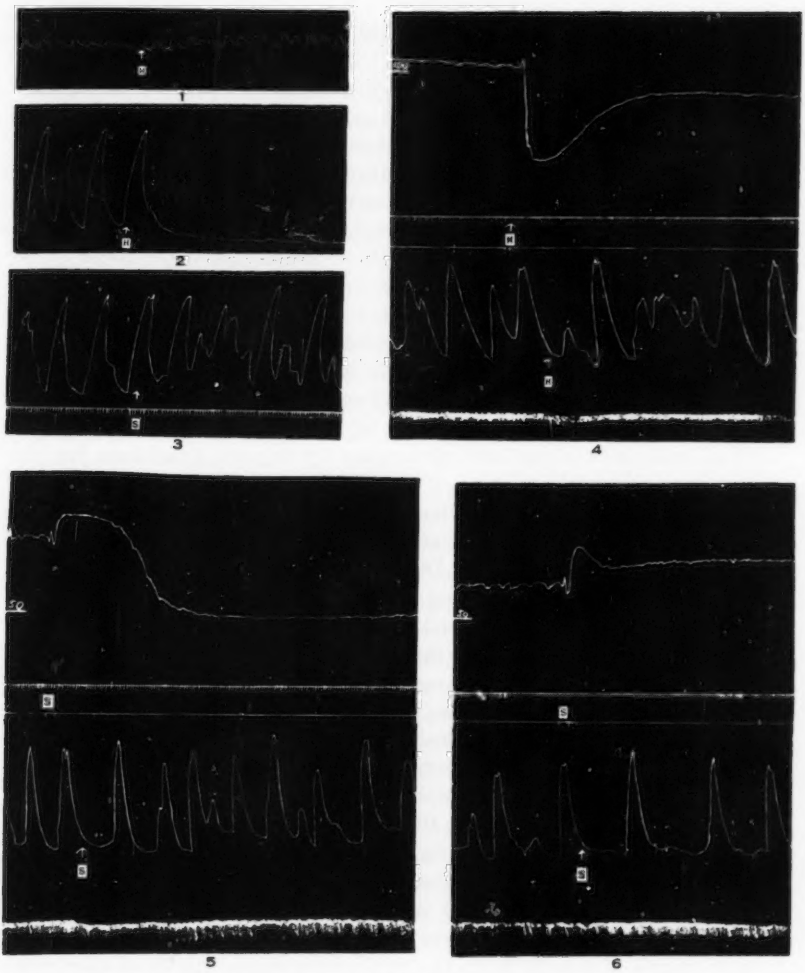

Fig. 1. In all figures time is recorded in five second intervals. Excised uterus, young rat. *H*, histamine, approximately 1:5,000,000.

Fig. 2. Suprarenalectomized rat with cortical transplants; oestrus II. Excised uterus. *H*, histamine, 1:400,000.

Fig. 3. Suprarenalectomized rat with cortical transplants; oestrus II; sensitized 10 days previously. Excised uterus. *S*, shocking dose of 0.5 cc. horse serum.

Fig. 4. In this, and in subsequent figures, upper record is blood pressure, middle record is uterine activity, lower record is respiration, and time record is at zero blood pressure level. Suprarenalectomized rat with cortical transplants, 200 grams; oestrus II. Uterus in situ. *H*, intravenous histamine, 0.05 mgm. per 100 grams.

Fig. 5. Suprarenalectomized rat with cortical transplants, 240 grams; oestrus II; sensitized 13 days previously. Uterus in situ. *S*, intravenous shocking dose of 1.0 cc. horse serum.

Fig. 6. Same animal as in figure 5. *S*, 1.0 cc. horse serum given 20 minutes later than *S* in figure 5; desensitization test.

1932). To preserve the normal activity gradient one entire horn with the ovary attached was set up. Moreover, as far as possible only mature rats in oestrus were chosen because of the improved rhythmicity of the contractions during this period (Blair, 1922; Harne, 1932).

Records of the intact uterus in situ were obtained under urethane anesthesia (0.15 gm. per 100 gm.) by attaching a silk thread from a light lever to the thin strand of connective tissue at the bifurcation of the horns. The uterus was protected from the pressure of the surrounding intestines by a small celluloid trough placed beneath it. The broad ligament rested upon the wide rolled lips of the trough so that there was no interference with circulation or injury to the tissue. The abdominal incision was sewed up except for a distance of from 0.5 to 1.0 cm. over the bifurcation, interference with the thread being prevented by a small wire retractor. By this method minimal trauma and approximately normal conditions for the uterus were obtained.

The femoral or external jugular vein and the carotid artery were cannulated for injections and blood pressure determinations respectively. Heparin (Hynson, Westcott & Dunning, 10 mgm. per rat) was injected intravenously to prevent clotting in the blood pressure experiments. A pneumograph record was procured by catching a few hairs of the thorax in a small clip attached by a silk thread to another light lever. All levers were arranged to write simultaneously on a smoked drum.

Histamine, ex situ. In a series of 24 normal, 16 doubly suprarenalectomized and 6 suprarenalectomized rats with cortical transplants, complete standstill of the rhythmically beating excised uterus or a prolonged period of diminished frequency and amplitude was regularly produced by histamine (Ergamine acid phosphate, Burroughs Wellcome & Co.) in concentrations of 1:200,000 to 1:500,000 (fig. 2). Although a dilution of 1:1,000,000 at times caused marked inhibition, there was frequently no appreciable response or one so slight that it was difficult to distinguish sufficient departure from the normal deviations to be sure of the reaction. Some variation in degree of reactivity to the amine was characteristic of specimens from both operated and unoperated animals. The three types of rats showed no essential differences with respect to the character, sensitivity or regularity of the reaction of the excised uterus; but the initial activity of the uterine muscle from rats with cortical insufficiency was somewhat erratic.

In contrast to the consistent inhibitory effect of histamine seen in the above cases, there were three instances in which the amine in a dilution around 1:5,000,000 repeatedly produced a slight but definite rise in tone (fig. 1). The specimens were obtained from normal rats. This response could not be attributed either to age, oestrus or to slight pH or temperature changes of the bath, since control experiments showed that these factors

failed to alter the nature of the reaction. It was not possible to obtain any other unquestionable examples of reversal because such slight variations did not always warrant interpretation as evidence of stimulation.

Anaphylaxis, ex situ. The actively sensitized uterine muscle from normal, suprarenalectomized, or "transplanted" rats responded to the shocking dose of antigen by a mild increase in tone and usually in frequency of the partial contractions, if any reaction at all was obtained (fig. 3). It was necessary to employ a relatively large amount of antigen, namely 0.5 to 1.0 cc. of serum. Nevertheless the responses obtained were unquestionably manifestations of anaphylaxis, as subsequent injections of the same or even greater amounts of serum would not provoke a similar reaction, conclusively illustrating the phenomenon of desensitization. Moreover in the control series of 20 cases the non-sensitized muscle was not similarly stimulated by the antigen. The number of positive cases obtained was as follows: 6 of 14 tests on normal rats, three of which were very slight; 2 of 5 tests on transplanted rats; 3 of 10 tests on suprarenalectomized rats.

The results of experiments on the excised uterus, as regards both histamine and anaphylactic reactions, support the previous opinions (Parker and Parker, 1924; Voegtlin and Dyer, 1925; Knaus and Clark, 1925; Kelloway, 1930) that the smooth muscle in this species is not a particularly sensitive or reactive tissue. The difficulty in eliciting an anaphylactic response of the excised rat uterus was not mitigated by previous removal of the suprarenal glands or suprarenal medulla, although it has been demonstrated that under these conditions the susceptibility of the animal is strikingly enhanced.

Histamine, in situ. Definite inhibition of the uterus in situ was not obtained after intravenous injections of histamine in amounts ranging from 0.01 mgm. to 10.0 mgm. per 100 grams body weight. Although an immediate, transient (30–45 seconds) and very slight drop of tonus occurred in the majority of cases, the effect was in no way comparable to that obtained with excised muscle. Moreover the same type and degree of relaxation could be produced by lowering the blood pressure by other means, such as vagus stimulation and amyl nitrite inhalation. The fleeting inhibition was simultaneous with the rapid fall in blood pressure and therefore may be attributed to a transient, inadequate circulation to the organ, as has been observed by Barbour and Rapoport (1922) in the dog.

A second phase of increased tonus followed immediately, accompanied at times by some increase in rate and amplitude of the contractions (fig. 4). Although this change was at best a relatively feeble reaction, it was more pronounced than the relaxation phase and sometimes lasted as long as 20 minutes. In the records from normal rats it was frequently accentuated by contrast with the period of inactivity preceding the injections.

As this failure to obtain an initial vigorous uterine rhythm was encountered only in the normal animals it may be attributed to the stimulation of adrenin secretion by the operative procedures (Cannon, 1931). Except for this feature the myographs of 17 normal, 12 doubly suprarenalectomized and 10 rats with cortical transplants did not show any distinct differences in the uterine response to the amine. The dominating and profound systemic disturbances with larger doses of the drug made it impossible to procure satisfactory uterine records.

Anaphylaxis, in situ. Control experiments on non-sensitized rats of all three types showed that horse serum had no vaso-depressor action and elicited no detectable change in the uterus.

In a series of 11 sensitized normal rats the occurrence of lachrymation and mild dyspnea following, in 6 cases, the shocking dose of antigen suggested a weak anaphylactic reaction. In one case this was accompanied by a vague stimulation of the uterus, while in a seventh case the increase in tone and frequency was definite; but other manifestations of shock failed to appear. In all instances recovery was rapid and complete.

In 9 of 11 rats with cortical transplants the symptoms of shock were more pronounced. All developed varying degrees of dyspnea, and on biopsy the gastro-intestinal tract of 7 showed characteristic petechial hemorrhages and bloody exudation into the lumen of the ileum. In 6 of these, 1 to 4 minutes after the introduction of antigen, both the tone of the uterus and number of partial contractions increased slightly for a period of several minutes. Three died in from 22 to 45 minutes.

In 10 doubly suprarenalectomized rats tested for shock a consistent finding was dyspnea, accompanied in most cases by acute gastro-intestinal symptoms. Six died in from 14 to 120 minutes. In three of the fatal cases, and in one that survived, definite uterine stimulation followed the test dose in from $\frac{1}{2}$ to $3\frac{1}{2}$ minutes. In the other fatal cases the uterine reaction was negative or indefinite.

A second group of experiments of this nature was carried out with blood pressure determinations in order to obtain a more conclusive demonstration of anaphylaxis. Furthermore, in all cases of survival, the ineffectiveness of a second injection of the serum on the blood pressure illustrated desensitization more conspicuously than in the case of the uterine musculature (fig. 6).

In $3\frac{1}{2}$ to 7 minutes after the test dose, the average initial blood pressure of 111.3 mm. Hg for 3 sensitized normal rats fell during the course of 10 minutes to an average level of 66.6 mm. Hg. In 2 cases a mild uterine stimulation occurred simultaneously with the fall in pressure. At the end of 30 minutes or less recovery was complete, and on biopsy no pathological changes were found. In a series of 9 rats with cortical transplants similar changes in blood pressure occurred in 8, the average drop being

from 104.0 mm. Hg to 47.5 mm. Hg. The uterus, in 6 instances, again showed evidence of a varying but slight degree of increased activity (fig. 5). One death occurred 30 minutes after the injection: in the survivors at the end of one hour no recovery had taken place in 4 cases, while in 3 the recovery was incomplete.

In 7 of a series of 8 doubly suprarenalectomized rats the average initial blood pressure of 75.1 mm. Hg fell to 48.0 mm. Hg in from 3 to 4 minutes after the shocking dose. Six died in from 32 to 131 minutes, and one recovered after 40 minutes. The uterus manifested a slight rise in tone and rate in 5 instances. Congestion of the gastro-intestinal tract was marked in all.

In addition, 3 normal rats, 2 doubly suprarenalectomized rats and 5 rats with cortical transplants were passively sensitized by intraperitoneal injections of 0.25 to 1.0 cc. of anti-ovalbumin-rabbit serum. Three and four days later the test dose of purified 1 per cent or 5 per cent ovalbumin was given by vein. A marked fall in blood pressure occurred in all instances. One doubly suprarenalectomized rat and three "transplants" died. A faint uterine reaction was observed in only 3 of the 10 cases. Control injections of ovalbumin into non-sensitized rats as well as desensitization tests, showed that the observed changes were truly anaphylactic in nature and not due to the slight toxicity of the antigen. Although the number of cases was limited, it was apparent that the use of a highly purified antigenic protein did not fundamentally alter the characteristic anaphylactic reactions of the rat—namely, the increased susceptibility of the animals lacking the adrenal medulla, and the comparative insensitivity of the uterine muscle.

It is apparent that the anaphylactic response of the uterine smooth muscle of the rat, when obtained, is always one of mild stimulation; very similar in character and degree to that occurring in situ after histamine. That the reaction is feeble and does not consistently reflect the intensity of the anaphylactic condition might be expected from previous experience with the excised muscle.

Discussion. The reversal of the uterine response in situ as compared with that ex situ may be due to a quantitative dosage factor, since it is likely that the actual amount of the amine acting on the uterine muscle is far less than the amount injected, it being rapidly inactivated by tissue or by tissue fluids (Best and McHenry, 1931; Percival and Scott, 1931). It has been shown moreover that the potency of other drugs administered intravenously may be less than the value calculated from the action on excised tissue (Knaus and Clark, 1925; Salant and Parkins, 1932). Other factors to be considered, in the alteration of the reactivity of the intact organ, are variations in the Ca:K ratio and in the H-ion concentration of the internal environment occurring in histamine and in anaphylactic shock

(Kuschinsky, 1929; Zimmermann, 1931; Berthelson and Murdick, 1931). The control experiments as well as the results of Salant and Parkins (1932) show that the excised rat uterus is little if at all affected by relatively wide differences of this nature. It is therefore unlikely that such changes in the internal environment would be of sufficient magnitude entirely to account for the observed results. In spite of the sporadic occurrence of stimulation of the excised uterus by high dilutions of histamine, it may be that the regularity of this reaction in situ signifies that it is the characteristic response to minute amounts.

The results reported above show that blood-pressure changes reveal the anaphylactic condition in rats much more consistently than does the uterine reaction. The increased susceptibility of the rat to histamine and to anaphylactic shock after removal of the suprarenal glands was not accompanied by a similar alteration in the sensitivity of the uterus. If a higher degree of sensitization had been induced by loss of the glands, it would be expected that the smooth muscle would reflect this difference by an increase in the number of positive responses and in the magnitude of the reaction. The results confirm, however, the conclusion of Flashmann (1925) and of Wyman (1929) that the degree of sensitization does not depend upon the presence or absence of the suprarenal glands. The circulatory collapse in anaphylaxis after suprarenalectomy is more likely the result of an inadequate defense mechanism (Wyman, 1929).

The recent work of Dragstedt and Gebauer-Fuelnegg (1932) has materially strengthened the histamine theory of anaphylaxis. They found that a toxic agent, chemically and pharmacologically resembling histamine, can be obtained from the thoracic lymph or vena-caval blood of the dog in anaphylactic shock. Confirmatory experiments of this nature on the guinea pig have also been reported by Bartosch, Feldberg and Nagel (1932). As stated above, the reaction of the isolated rat uterus has been pointed out as a singular discrepancy in the histamine theory. In view of the fact that the uterus in situ is stimulated, after intravenous injections of histamine, in a fashion very like that observed in anaphylaxis, this criticism loses much of its significance.

SUMMARY

1. In the rat, histamine depresses the spontaneous activity of the excised uterus in dilutions up to 1:1,000,000.

2. Intravenous injection of histamine from 0.01 mgm. to 10.0 mgm. per 100 grams body weight, does not produce inhibition in situ similar to that obtained with excised muscle, but appears to cause a mild stimulation of tone and frequency.

3. The response of the rat uterus to anaphylactic shock, in situ and ex situ, is one of mildly increased activity.

4. The predominant reaction both in anaphylactic shock and in histamine shock is in the rat the fall in blood pressure.

5. Double suprarenalectomy, with or without autoplastic cortical transplantation, decreases the resistance of the rat to both types of shock without, however, appreciably altering the reactivity of the uterine muscle.

6. The similarity between the uterine reactions in situ, to histamine and to the factors in anaphylactic shock indicates that evidence against the liberation of histamine or a histamine-like substance in anaphylaxis, cannot be derived with certainty from the difference in the responses of the excised rat uterus to both these conditions.

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THE EFFECT OF HISTAMINE ON THE BLOOD SUGAR IN SUPRARENALECTOMIZED RATS

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According to previous investigators the administration of histamine results in hyperglycemia (Chambers and Thompson, 1925; La Barre, 1926; Menten and Krugh, 1928). Katzenelbogen and Abramson (1927) found that in severe histamine shock there is a hypoglycemia, while in mild shock there is a hyperglycemia. There has been some question as to whether the hyperglycemia is due to a more specific event than concentration of the blood (Best and McHenry, 1931, p. 432). La Barre attributed it to stimulation of the vagus fibres in the liver.

It is known that histamine stimulates secretion of the suprarenal medulla. Moreover suprarenalectomized rats, with or without cortical tissue, are about 20 times more susceptible to histamine shock than normal rats (Wyman, 1928; Wyman and tum Suden, 1932). It seemed therefore worthwhile to study the effect of histamine on the blood sugar level in such animals.

METHODS. The general technique of studying suprarenal insufficiency in the albino rat has been described in previous papers of this series. Three types of animals were used; unoperated, suprarenalectomized (from 2 to 7 weeks after operation), and suprarenalectomized with autoplastic transplants of cortical tissue but no demonstrable medullary tissue (from 10 to 23 weeks after transplantation). Males and females were chosen in approximately equal numbers and no sex correlations were observed in the results. Ergamine acid phosphate (Burroughs, Wellcome & Co.) dissolved in sterile physiological saline solution was injected intraperitoneally. Blood sugar was determined by the micro-method of Folin and Malmros (1929) in 24-hour fasting rats. Determinations were made 30 minutes before and 10 and 30 minutes after the injection of histamine or physiological saline solutions. Blood samples were taken from the tip of the tail without anesthesia (see Wyman and Walker, 1929). Per cent of blood plasma was determined by hematocrit.

[1] The authors gratefully acknowledge the assistance of Solomon J. Gootman, with the blood sugar determinations.

RESULTS. The results are summarized in table 1. In normal rats the blood-sugar values varied from 74.2 to 108.8 mgm. per 100 cc. whole blood, averaging 91.8 mgm. (39 determinations). In rats having transplanted cortical tissue but no medullary tissue the blood sugar varied from 62.5 to 111.5 mgm., averaging 79.0 mgm. (35 determinations). In suprarenalectomized rats showing no marked symptoms of suprarenal insufficiency the blood sugar varied from 59.0 to 88.5 mgm., averaging 71.9 mgm. (20

TABLE 1

INJECTIONS. SALINE SOL. OR HISTAMINE, MGM. PER 100 GMS.	NO. OF EXPTS.	NO. WITH NO SIGNIFICANT CHANGE	NO. WITH RISE IN BLOOD SUGAR	NO. WITH FALL IN BLOOD SUGAR	AV. BL. SUGAR 30 MIN. BEFORE INJ.	AV. BL. SUGAR 10 MIN. AFTER INJ.	AV. BL. SUGAR 30 MIN. AFTER INJ.	PER CENT CHANGE IN AV. AFTER 10 MIN.	PER CENT CHANGE IN AV. AFTER 30 MIN.
Normal rats									
					mgm. per cent	*mgm. per cent*	*mgm. per cent*		
Saline ½ or 1 cc	9	8	1	0	88.1	87.8	87.3	0	0
Hist. 2 mgm.	9	4	5	0	90.3	98.8	107.5	+9.4	+19.0
Hist. 3 mgm.	6	1	5	0	99.7	108.1	115.5	+8.4	+15.8
Hist. 5 mgm.	8	2	6	0	94.3	103.0	109.2	+9.2	+15.8
Hist. 10 mgm.	7	1	6	0	93.1	118.2	118.2	+26.9	+26.9
Autoplastic cortical transplants									
Saline sol. 1 cc.	12	12	0	0	77.1	77.9	77.2	0	0
Hist. 0.1 mgm.	9	8	0	1	76.2	74.2	74.3	0	0
Hist. 0.25 mgm.	6	2	0	4	76.5	70.7	68.2	−7.6	−10.8
Hist. 0.5 mgm.	3	0	0	3	94.1		72.3		−23.2
Hist. 1 mgm.	5	0	0	5	82.1	74.9	68.7	−8.8	−16.3
Doubly suprarenalectomized rats									
Saline sol. 1 cc.	7	7	0	0	71.8	70.9	70.7	0	0
Hist. 0.1 mgm.	4	4	0	0	76.9	78.3	76.2	0	0
Hist. 1 mgm.	8	0	0	8	72.1	66.9	50.9	−7.2	−29.4

determinations). These figures agree well with those obtained in previous investigations (see Wyman and Walker, 1929).

Following control injections of physiological saline solution there were, in all three types of rat, no significant changes in blood sugar or in per cent of blood plasma. In unoperated rats hyperglycemia occurred after the injection of from 2 to 10 mgm. of histamine per 100 grams body weight; this being as much as 40 per cent of the original blood sugar value in numerous individual instances. The per cent of blood plasma had fallen slightly 10 minutes after the injection, but not nearly enough to account

for the hyperglycemia on the basis of blood concentration; 30 minutes after injection, when the hyperglycemia was most marked, the per cent of plasma had returned to normal or just below normal.

In transplanted and suprarenalectomized rats the injection of 0.1 mgm. of histamine per 100 grams resulted in no significant changes in blood sugar and only a slight fall in per cent of plasma, which quickly recovered. Hypoglycemia followed the injection of 1 mgm. of histamine per 100 grams, the drop being around 24 per cent of the original blood sugar value in several cases of the transplants, and as much as 54.2 per cent in one of the suprarenalectomized rats. In these animals the per cent of blood plasma fell somewhat and did not recover entirely 30 minutes after injection, so that the blood sugar determinations may have been somewhat high. The greater degree of hypoglycemia after histamine in the suprarenalectomized rats as compared with that in the transplants is probably associated with the fact that the liver and muscle glycogen stores are depleted in suprarenal insufficiency (Britton and Silvette, 1932).

Discussion. The hyperglycemia following small doses of histamine in unoperated rats is real: it is not a reflection of blood concentration. Apparently it is mediated by the suprarenal medulla, as might be expected since histamine stimulates secretion of this gland. This conclusion is drawn because, in suprarenalectomized and in transplanted rats, there is no significant change in blood sugar following smaller doses of histamine (which are probably comparable in these more susceptible animals to the larger doses in normal rats), and hypoglycemia following larger doses. In so far as severe vascular disturbances follow even relatively small doses in suprarenalectomized rats, our results are consistent with those of Katzenelbogen and Abramson (1927). Hypoglycemia appears to accompany severe histamine shock, whether due to high histamine dosage or to lack of the suprarenals.

The striking similarity between the symptoms of suprarenal insufficiency and those of traumatic shock has long been recognized. In a recent summary as many as 31 resemblances were listed (Swingle, Pfiffner, Vars, Bott and Parkins, 1933). One dissimilarity has been emphasized by other authors, namely, that the blood sugar is decreased in cortical insufficiency but is normal or elevated in shock (Freeman, 1933). If the histamine theory of traumatic shock advocated by Cannon and Bayliss is true, hyperglycemia in a shocked animal possessing its suprarenal medulla might be expected. In suprarenalectomized animals, on the other hand, hyperglycemia may be absent because of concomitant lack of the suprarenal medulla; hypoglycemia is present since the cortex is concerned with the maintenance of a normal blood sugar level. There is also some evidence that histamine or histamine-like toxic substances accumulate in suprarenal insufficiency (Banting and Gairns, 1926; Lucas, 1926). These would

contribute to the hypoglycemia. The apparent discrepancy therefore becomes explainable and suprarenal insufficiency may still be spoken of as a "shock-like condition."

SUMMARY

Hyperglycemia follows the injection of small doses of histamine in normal rats, while hypoglycemia follows the injection of comparable doses in suprarenalectomized rats with or without transplanted cortical tissue. The hyperglycemia is apparently mediated by the suprarenal medulla.

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THE RELATION BETWEEN BLOOD PRESSURE, BLOOD UREA NITROGEN AND FLUID BALANCE OF THE ADRENALECTOMIZED DOG[1,2]

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Marshall and Davis (1916) first called attention to the elevation of the blood urea and non-protein nitrogen of the adrenalectomized animal exhibiting symptoms of insufficiency. Their observations have since been repeatedly confirmed (Rogoff and Stewart, 1926; Banting and Gairns, 1926; Lucas, 1926; Swingle, 1927; Hartman, MacArthur, Gunn, Hartman and MacDonald, 1927; Wyman and Walker, 1929; Silvette and Britton, 1933; Harrop, Pfiffner, Weinstein and Swingle, 1932, and others). The last named authors utilize the blood urea nitrogen changes following adrenalectomy as the basis for standardizing adrenal cortical extracts.

So far as the writers are aware, but three groups of investigators appear to have studied experimentally the problem of the increase in blood non-protein and urea nitrogen following adrenalectomy in relation to the blood pressure, and those who have done so, namely, Marshall and Davis (1916), Swingle, Pfiffner, Vars, Bott and Parkins (1933) and Harrop and Weinstein et al. (1933) are not in agreement.

EXPERIMENTAL METHODS. The type of adrenalectomized animal used in the experiments has been adequately described in earlier papers of this series of adrenal studies (Swingle, Pfiffner, Vars, Bott and Parkins, 1933). The dogs were carefully trained for blood pressure work, and the arterial pressure determined in the femoral artery with the Dameshek and Loman instrument (Dameshek and Loman, 1932; Parkins, 1934). The blood urea nitrogen was determined by the Van Slyke (1929) manometric (hypobromite) method, and hemoglobin by the method of Newcomer. The animals were maintained in normal health (see footnote 3 in previous papers of this series of adrenal studies) by adequate daily injections of

[1] In the collaborative studies on the adrenal cortex carried on in this laboratory the writers have specialized in certain phases of the experiments. The physiological aspect of the problem is the responsibility of the senior author (W. W. S.); the biochemical work of extraction and purification of the hormone that of one of us (J. J. P.).

[2] These investigations have been aided by a grant from the Josiah Macy Jr. Foundation, New York.

cortical hormone until ready for experimentation. The extract was withheld and the dog permitted to develop severe symptoms of insufficiency and then revived from the terminal prostration and restored to normal health by hormone administration. The blood pressure and urea nitrogen were followed daily, and in several animals twice daily.

Relation of the blood urea nitrogen to changes in arterial pressure. Marshall and Davis (1916) state that the blood urea nitrogen changes in their experimental cats were independent of the arterial pressure and that the elevation in urea occurs when the blood pressure is normal. They concluded that the adrenal secretes some substance necessary for maintenance of normal kidney function.

Harrop, Weinstein et al. (1933) concur in this view, stating that the accumulation of the blood urea nitrogen can not be merely secondary to circulatory failure and fall in blood pressure since (according to their data) collapse of the circulation with fall in blood pressure takes place only after the rise in non-protein and urea nitrogen is well established.

The present writers (1933) in a preliminary paper pointed out that the elevation of blood urea in the adrenalectomized dog deprived of cortical hormone, occurs simultaneously with the fall in arterial pressure and presumably capillary filtration pressure in the kidney, and that an inverse ratio exists between blood pressure and waste nitrogen.

These contradictory results of the various investigators are more apparent than real and are probably due (in our opinion at any rate) to 1, the accuracy of the blood pressure method employed (Harrop and Weinstein et al. modified cuff method in the unanesthetized dog), and 2, the anesthetic and training of the experimental animal, and suitability of the method for the purpose (Marshall and Davis, using a local anesthetic, and arterial cannulation in untrained adrenalectomized cats).

The data collected in this laboratory are summed up in plate I, in which the blood pressure and urea nitrogen changes of six adrenalectomized dogs are graphically represented. The first part of the insufficiency cycle, i.e., following extract withdrawal to the time of onset of severe symptoms, is the important part, in so far as the relation of blood pressure to blood urea is concerned; consequently the recovery phase of the cycle has been omitted from the graphs of dogs 122, 88 and 55. Dogs 151 and 61 show one complete cycle (insufficiency and recovery on extract). Dog 52 shows two complete cycles. With the exception of animals 52 and 61, the blood pressure and blood urea were followed daily. The cases are representative of the twenty animals studied in regard to this particular point, plate I. Dog 61 was used for a hemorrhage experiment during the second insufficiency cycle.

It is obvious that an inverse ratio exists between blood pressure and blood urea nitrogen in the adrenalectomized dog. *The blood urea rises*

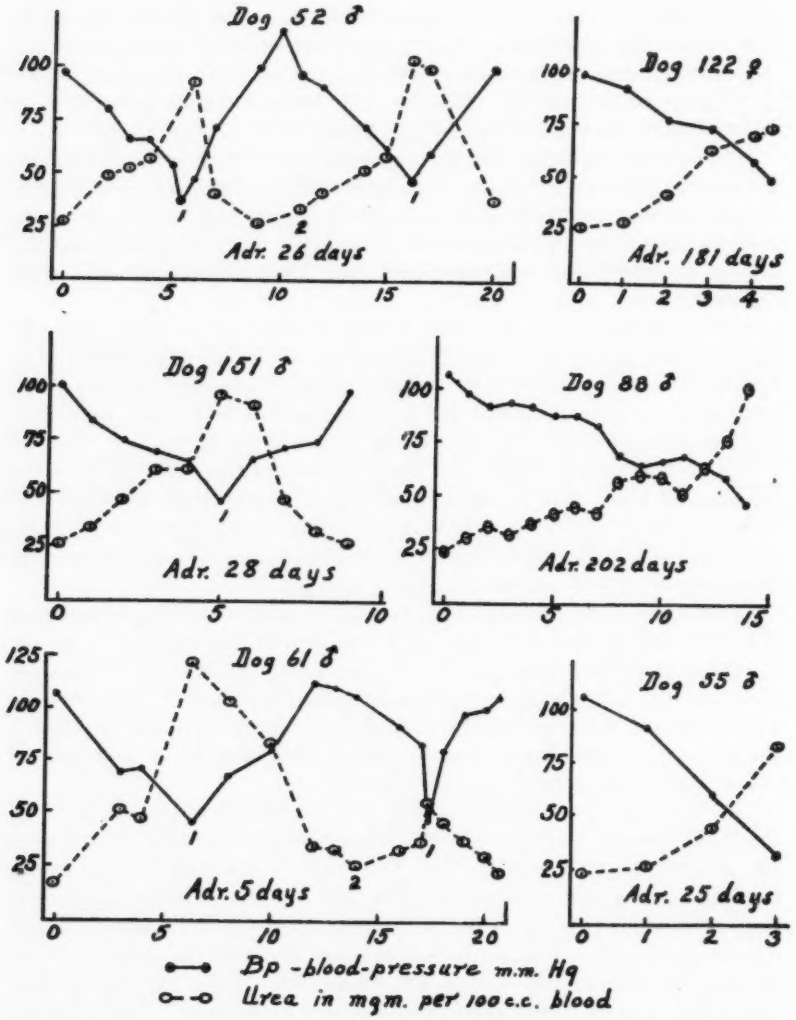

Plate I. The inverse relation between blood pressure and blood urea nitrogen in the adrenalectomized dog deprived of cortical hormone. The blood pressure and urea were determined daily in dogs 122, 88, 55 and 151. Animals 52 and 61 were studied through two complete cycles of insufficiency and recovery. Dog 61 was used for a hemorrhage experiment during the second insufficiency cycle. *1* = point when hormone was injected intravenously; *2* = point when hormone injections were discontinued; *Adr.* = interval in days between bilateral adrenalectomy and use in the experiment.

simultaneously with the fall in arterial pressure and does not precede it. A glance at any of the graphs (plate I) reveals this fact clearly.

So closely do the changes in blood pressure and blood urea follow one another in inverse relationship through the cycle of insufficiency and recovery on hormone treatment, it is a simple matter to follow the arterial pressure and accurately predict the first elevation in urea, or conversely by determining the urea to predict the first fall in arterial pressure.

It is probable that the changes in blood pressure, as determined by the Dameshek and Loman instrument in the well trained unanesthetized adrenalectomized dog, can be utilized for purposes of bioassay of the cortical hormone.

Difference of opinion exists among various investigators as to whether or not significant anatomical changes occur in the kidneys of animals deprived of their adrenal glands.

Marshall and Davis (1916) and Swingle (1927) report no significant histological changes in the kidneys of adrenalectomized cats. On the other hand, Gunn, Cori and Hartman (1928) describe changes in the kidneys of adrenalectomized guinea pigs and rats dying of cortical insufficiency, which they regard as lipoid nephrosis. Marine (1928) also reported lipoid nephrosis as occurring in adrenalectomized dogs, cats, rabbits and rats. MacMahon and Zwemer (1929) have described the presence of unusually large amounts of fat in the convoluted tubules of adrenalectomized cats. Banting and Gairns (1926) studied the kidneys of dogs succumbing to adrenal insufficiency, and described general hyperemia with small focal areas of hemorrhage throughout, cloudy swelling of the tubules, and the presence of albuminous casts.

In view of such conflicting evidence it is impossible to arrive at a satisfactory conclusion. However, it appears unnecessary to search for, or expect to find, significant renal changes, since it is probable that the azotemia of adrenal insufficiency is extra-renal in origin and may have no necessary relation to kidney failure. The nitrogen retention appears to be due to the operation of factors similar to those which lead to retention in other morbid states where dehydration and base and chloride deficit occur; e.g., pyloric stenosis, experimental high intestinal obstruction, paralytic ileus, external drainage of pancreatic secretions, and infantile anhydremia. The question of direct renal or adrenal cortex involvement does not necessarily enter in these conditions so far as known at present.

Harrop, Weinstein et al. (1933) have pointed out the reciprocal relation existing between the sodium and chloride and the urea nitrogen following extract withdrawal. It is apparent that an inverse relation exists between urea nitrogen on one hand, and the blood pressure and electrolytes on the other. It has been suggested by Blum (1929), Hartmann and Darrow (1928), Rachmilewitz (1934) and others, that in azotemias of extra renal

origin, accompanied by base and chloride deficit and dehydration, the urea elevation may be related to the decrease in electrolytes. A significant relation between fall in electrolyte concentration and increase in blood urea undoubtedly occurs in adrenal insufficiency. It is highly probable also, that during insufficiency and the consequent dehydration and shock, abnormal destruction of tissue protein occurs. This would account for part of the urea increase but by no means all of it. The reciprocal relation between blood urea elevation on one hand and the decrease in electrolytes, fluid volume and arterial pressure on the other hand, seems not without significance. The blood pressure itself probably merely reflects the fluid volume and electrolyte changes following withdrawal or injection of cortical hormone. It should be pointed out in this connection, that the urea nitrogen can be lowered and kept at a normal level by supplying the dog in insufficiency with enough fluid to stimulate marked diuresis.

The fluid balance of the adrenalectomized dog. Loeb et al. (1933) first called attention to the increased daily urine volumes of the adrenalectomized dog coming into insufficiency as compared with the output during the same time interval previous to adrenalectomy. The data as presented by these investigators record only the urine volumes. Harrop, Weinstein et al. (1933) have, however, given complete data on the water balance of their dogs.

The water balance of six adrenalectomized dogs (eight experiments, since dogs 88 and 99 were used twice) are graphically represented in plate II. Three of these dogs 88, 99, and 151 were studied through the complete cycle, i.e., the fore period before extract withdrawal, the cycle of insufficiency and the recovery phase on extract treatment. Table 1 gives the data on dog 151, a typical case.

It will be observed at once that great variation as to the daily total food and water intake and urine output occurred among the animals. In four dogs, nos. 122, 144, 120 and 99b (plate II) the average daily urine volumes during the period of insufficiency were actually less than during the fore period when the animals were enjoying normal health; on the other hand, dogs 88 and 99a and 151 excreted greater average daily urine volumes during the period of insufficiency than they did during the fore period. Dog 99a (the same animal used twice during a twelve month interval) at one time excreted more urine during insufficiency than he did during the control period, and in a later experiment (99b) excreted less. In all cases, however, *in relation to the total food and water intake*, the animals put out daily urine volumes after withdrawal of cortical hormone, in excess of the volumes excreted during the fore period. In other words, the adrenalectomized dog off extract may excrete less urine than he did during the same time interval during the fore period (50 per cent of the animals do so) but since he invariably takes in less food and water, the ratio of fluid output to total

fluid intake is always greater. The dog exhibits a negative water balance in this respect (86 cc. daily in the case of dog 151).[3]

It is evident from the figures on daily water output in relation to intake as presented in table 1 and plate II and summarized in table 2 that the loss of body fluids (in relation to intake) by way of the urine is quite insufficient to account for the observed decline in blood and plasma volume and con-

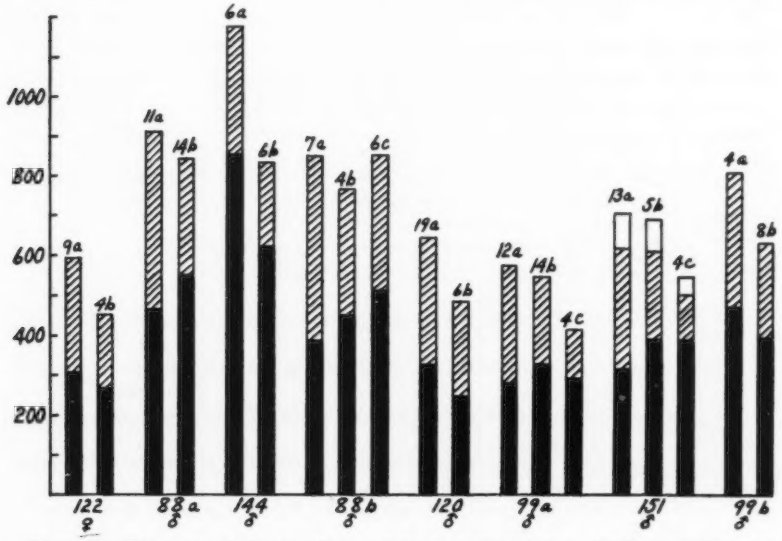

Plate II. The water balance of six adrenalectomized dogs (eight experiments). Total height of the columns = average daily fluid intake in cubic centimeters; black columns = average daily urine volumes in cubic centimeters; letters at top of column = *a*, control period; *b*, insufficiency; *c*, recovery on hormone treatment; numerals at top of column = number of days in each period. Dogs 88, 99, and 151 were each studied through one complete cycle of insufficiency and recovery. The remainder of the animals were observed only through the control and insufficiency periods. Metabolic water has been calculated for dog 151 and indicated on the plate by the clear block at the top of the columns.

sequent fall in arterial pressure of the adrenalectomized dog following withdrawal of cortical hormone.

[3] The total fluid intake and output of the dogs used in the experiments (a typical detailed example of which is illustrated by dog 151, table 1 and fig. 2) was calculated according to the principle of Babcock (1912). The composition of the diets used is given by the manufacturers of Ken-L-Ration and Bal-Ra. The metabolic water calculations were multiplied by the digestibility factor 0.9 given by Bing and Mendel (1931). The water lost by evaporation, expired air and feces was not determined. Diarrhea and vomiting did not occur.

A normal dog of the same weight as no. 151, with adrenals intact, and deprived of all food and water for five days maintains himself in normal condition despite the elimination of far more fluid (as urine) than 430 cc. during this interval.

TABLE 1

Complete data on the water balance, blood pressure, hemoglobin and urea of a representative animal

Dog 151. Male

	DATE	WEIGHT	FOOD INGESTED	H_2O IN FOOD	H_2O DRANK	TOTAL H_2O INTAKE	URINE VOLUME**	PULSE	BLOOD PRESSURE	UREA	HEMOGLOBIN	REMARKS
		kgm.	*gm.*	*cc.*	*cc.*	*cc.*	*cc.*					
Control period*	Dec. 29/33	11.4	500	350	165	515	290					9th day of control period
	Dec. 30/33	11.3	600	470	310	780	280					
	Dec. 31/33	11.4	600	470	460	930	370					
	Jan. 1/34	11.5	600	470	205	675	465					
	Jan. 2/34	11.6	600	470	280	750	360	80	101			
Insufficiency	Jan. 3/34	11.6	600	470	180	650	470	80	100	26	13.6	Extract discontinued
	Jan. 4/34	11.5	500	350	230	580	410	144	83	34	14.6	Appears perfectly normal
	Jan. 5/34	11.3	506	350	290	640	340	152	74	47	14.8	Vigorous and playful
	Jan. 6/34	11.3	420	294	470	764	410	136	69	61	14.6	Active and playful
	Jan. 7/34	11.3	310	217	205	422	310	132	64	61	15.4	Mild symptoms
Recovery	Jan. 8/34	11.1	0	0	230	230	330	76	46	97	16.0	Marked symptoms; 33 cc. extract injected 9:00 a.m.
	Jan. 9/34	10.8	155	108	330	485	570	144	66	91	14.8	Somewhat weak but playful
	Jan. 10/34	10.6	235	164	270	435	280	148	71	47	13.7	Active and playful
	Jan. 11/34	10.5	500	350	360	710	360	140	74	32	14.2	Active and playful
	Jan. 12/34	11.0	500	350	280	630	365	84	98	27	13.6	Normal condition

* The control period was a 13 day interval. Only the last 5 days preceding withdrawal of extract are given in the table.

** The dogs were not catheterized but at the end of each phase of the cycle care was taken that significant amounts of urine did not remain.

Study of the water balance of the dog during the recovery phase of the insufficiency cycle (plate II and tables 1 and 2) reveals at once that during this phase while the animal is recovering, and the blood volume and arterial pressure are steadily rising, the hemoconcentration and urea nitrogen

decreasing, the dog is actually eliminating far more fluid as urine in relation to total fluid intake than he does during insufficiency (table 2). The fact of the matter is, the greatest loss of fluid in relation to intake occurs during the recovery phase after extract injection. This is to be expected since the hormone renders mobile all the available fluids of the organism. The writers wish to emphasize that loss of fluid by way of the urine is a factor in the dehydration of adrenal insufficiency, but not the sole factor and probably not the most important one.

According to the data collected in this laboratory on water balance and fluid exchange in the adrenalectomized dog off extract, two factors are chiefly responsible for the decline in blood and plasma volume and arterial pressure, and the general hemoconcentration and dehydration of adrenal insufficiency: 1, the animal exhibits a negative water balance and loses

TABLE 2

Summary of the water balance of dog 151

	AVERAGE DAILY WATER INTAKE PLUS MET. H_2O	AVERAGE DAILY URINE VOLUME	TOTAL INTAKE MINUS URINE VOL. (DAILY)	DAILY NEGATIVE BALANCE*	TOTAL NEGATIVE BALANCE
	cc.	*cc.*	*cc.*	*cc.*	*cc.*
Control period (13 days)	703	317	+386	Control used as standard for comparison*	
Insufficiency period (5 days)	688	388	+300	−86	−430
Recovery period (4 days)	543	385	+158	−224	−896

* The negative balances are the differences between retention in the normal control period and the periods of insufficiency and recovery.

more fluids from the body than he takes in; 2, the normal fluid exchange between blood stream and tissues is upset—transudation in the opposite direction, i.e., from tissues and interstitial tissue spaces to blood, is impaired, since fluid and salt are not freely mobile. These two factors taken together explain satisfactorily most if not all of the observed phenomena of adrenal insufficiency. Taken singly either factor is inadequate to serve as an explanation. The loss of fluid through the kidney alone is insufficient to account for the observed symptoms, or the fact that certain tissues and organs such as the skin, liver, muscles, and skeleton may be normal as to water content, or even more hydrated when the animal is dying of adrenal insufficiency than they are in the normal animal (Hartman et al., 1932; Silvette and Britton, 1933; Harrop, Weinstein et al., 1933). Moreover the phenomena following slight hemorrhage (study 1 of this series, 1934), the revival of the prostrate animal deprived of fluid and salt, the increased

urine output of such animals following massive injections of cortical hormone (study 2 of this series, in press), the continued excess of fluid output over intake during the recovery phase of the insufficiency cycle on extract treatment (table 1, plate II, dog 151), are inexplicable on any such idea. The fluids which are redistributed within the body of the adrenalectomized dog following hormone injections (animal not receiving fluid and food) can have but one source, the tissues and interstitial spaces.

SUMMARY AND CONCLUSION

1. Twenty adrenalectomized dogs were studied with regard to the relation of the blood urea nitrogen and the blood pressure. An inverse relation exists between the two throughout the cycle of insufficiency and recovery on hormone treatment.

2. The elevation of the urea nitrogen occurs simultaneously with the fall in arterial pressure and does not precede it.

3. The azotemia of adrenal insufficiency is probably extra renal in origin and may have no necessary relation to the kidney. The phenomenon is not peculiar to adrenal insufficiency but is common to those other morbid states where dehydration and base and chloride deficit occur. It appears to have a significant (but ill understood) relation to the changed electrolyte pattern.

4. The fall in arterial pressure in insufficiency is itself probably merely a reflection of the decline in volume of circulating fluid and associated electrolyte changes.

5. The water balance of six adrenalectomized dogs was studied. The animals not receiving cortical hormone excrete daily by way of the kidney a greater volume of water in relation to the total fluid intake, than they do during the control period.

6. The amount of fluid lost as urine (over total fluid intake) is inadequate to account for the dehydration and associated changes of adrenal insufficiency. A representative case, for example, lost but 430 cc. (over total fluid intake) during the entire insufficiency cycle.

7. A much greater loss of fluid (over intake) occurs during the recovery phase of the cycle, following injections of cortical hormone (896 cc. as compared to 430 cc. in a representative case) than the animal loses during insufficiency.

8. If the negative water balance of the animal in insufficiency is considered in conjunction with the fact that in such animals the mechanism for mobilization of fluid and salt, i.e., blood dilution, is lacking or greatly impaired, an adequate explanation is afforded for the observed phenomena. During the recovery phase, although the fluid loss is greater than during insufficiency, the fluids (and electrolytes) of the tissue and interstitial spaces are freely mobile.

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THE RELATION OF BILATERAL SUPRARENALECTOMY AND SUBSEQUENT EXTRACT THERAPY ON THE BODY WEIGHT AND OESTRUAL CYCLE OF THE ALBINO RAT[1]

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Within the past few years, there has been much disagreement among investigators concerning the effects of the removal of the suprarenal glands in the albino rat. Thus Lewis (13), del Castillo (4) and Schiffer and Nice (18) reported that only a small percentage of their animals showed glandular insufficiency, oestrous disturbance and death. However, Kitagawa (11), Wyman (20), Pencharz (16), Kutz (12), and Adler (1) have demonstrated that the majority of the rats do not survive, and in addition Martin (14a), Gaunt (8a), Firor and Grollman (5) and Corey and Britton (3b) agree that the oestrous cycles of most of their animals are definitely disturbed.

It is now well recognized that other consequences follow bilateral suprarenalectomy. The gradual loss of body weight is one which is significant and perhaps a suitable criterion of progressive cortical insufficiency. No extensive work has been reported concerning this effect of bilateral adrenalectomy in rats other than a brief mention of it by Gaunt (8a), Pencharz (16) and a few others. We have therefore undertaken a study of this point not only because it is an important result of suprarenalectomy but in the hopes that the return to normal of the body weight and oestrual cycle might serve as a criterion for experiments with extracts. Such a procedure would also serve to test the contentions recently made by Connor (2) that suprarenal cortical extracts contain an ovary inhibiting substance and by Corey and Britton (3a) that such extracts possess a specific sex stimulating principle. A preliminary report of this paper has been published (14b).

PROCEDURE AND RESULTS. A. *The effect of bilateral suprarenalectomy following a "two stage" operation.* Daily body weights and vaginal smears were recorded on a series of 35 adult female rats three to six months old. After a preliminary period during which the animals showed at least three normal successive oestrous cycles and increasing body weight, bilateral

[1] This problem was aided in part by grants from the Committee for Problems of Sex, National Research Council, administered by Dr. F. L. Hisaw.

suprarenalectomy was performed in two stages with a period of 4 to 21 days between the removal of the first and second glands. At the time of the removal of the second gland the body weights of the series ranged from 156 to 250 grams based on a three day pre-operative average. All operations were done under the ordinary antiseptic precautions and the technique and post-operative care similar to that reported in an earlier publication (14a).

1. *Effect on weight loss and survival* (table 1). The per cent weight loss based on a three day pre-operative (second operation) average was calculated for each of the 35 animals. Of these, 33 or 94.2 per cent died in a period of 6 to 29 days, the average being 14.9 days, following the removal of the second gland. The weight loss of those that died ranged from a minimum of 12.1 per cent to a maximum of 35.1 per cent, with an average of 21.1 per cent for the series. It was not always possible to obtain the weight of the animals at death, for often the rats died during the night. The last weight recorded on the living animal was therefore taken as the basis for the calculations. Weights on six of the rats were obtained at the point of death and twelve and twenty-four hours before death and in none of the cases did the decrease, if any, exceed two or three grams from the 24 hour record. Two of the 35 animals of the series survived, and when autopsied on the fifteenth and seventy-sixth post-operative days respectively, microscopic rests of cortical tissue were found in each case. At this time the weight of the former was six grams above the pre-operative average, and its greatest loss of weight was 12.6 per cent by the fourth day following the second operation. The other one of the two weighed 44 grams above the pre-operative average when killed and experienced a maximum weight loss of 6.2 per cent recorded by the eighth day.

The control series of female rats kept under the same conditions as the experimental ones consisted of five normal animals and fifteen other controls receiving the following treatment: 10 were unilaterally suprarenalectomized; 5 were successively splenectomized, unilaterally suprarenalectomized, unilaterally ovariectomized and hysterectomized in three different operations within a 15 day period. In all cases the animals survived. During the period that the bilaterally suprarenalectomized rats were losing weight and some dying from cortical insufficiency the normal unoperated controls showed an increase of 10 to 32 grams. The two groups of operated controls showed a drop varying from four to twelve grams after each operation, but the weight rose again on the second or third day and then gradually increased.

2. *Effect on the oestrous cycle.* The removal of one suprarenal had no appreciable effect on the oestrous cycle in both the experimental and control series other than a lengthening of the interval to the next oestrus in one of the experimental rats. However, the removal of the remaining

gland definitely disturbed the oestrous cycle in 91.4 per cent of the 35 animals. Complete absence of oestrum occurred in 14, 17 came into one oestrus followed by complete inhibition, one experienced two prolonged cycles followed by 16 days out of heat, and one came into several normal cycles. The two surviving rats showed normal or somewhat extended cycles. No observable oestrual disturbance was noted in any of the control animals including the series undergoing the three successive operations previously described. It is apparent bilateral suprarenalectomy has a definite effect on the oestrous cycle in over 90 per cent of the cases.

B. *The effect of bilateral suprarenalectomy following a "one stage" operation* (table 1). The general procedure, similar to that employed for the preceding series, was used on 33 adult females except that both glands were removed at one operation. Three animals were operated on with

TABLE 1

Effect of bilateral suprarenalectomy on the oestrous cycle, body weight and survival of adult albino rats

All were virgin females four to six months old

TYPE OF OPERATION	NUMBER OF RATS USED	PER CENT OESTROUS DISTURBANCE	PER CENT BODY WEIGHT LOST	PER CENT THAT DIED	SURVIVAL PERIOD OF THOSE THAT DIED	REMARKS
"Two stage"	35	91.4	12.1–35.1 Av. 21.1	94.2	6–29 days Av. 14.9	Accessory tissue found in 2 that survived
"One stage"	33	93.7	14.5–33.6 Av. 17.0	93.7	7–28 days Av. 11.7	Accessory tissue found in 2 that survived
Totals and averages..	68	92.5	19	93.9	13.3 days	

rigid surgical aseptic technique, and the others by the ordinary antiseptic method. The three day pre-operative weight average of the rats ranged from 159 to 243 grams.

1. *Effect on weight loss and survival.* Of the 33 rats operated upon, 31 or 93.7 per cent died in 7 to 28 days following the removal of the glands. The average survival was 11.7 days. The per cent weight loss of each rat was calculated as in the case of the first series reported. The maximum loss was 33.5 per cent and the minimum 14.5 per cent, the average for the 31 animals being 17 per cent. Two rats of the series survived, showed normal weight increase after a temporary decline and were later killed. In each case the maximum weight loss was recorded on the day after the operation. This decline amounted to 5.4 per cent and 7.4 per cent of the three days' pre-operative average. Tissue removed from both rats at post

mortem was fixed and sectioned and found to contain cortical rests. The control series for this "one stage" group consisted of seven normal females and four females successively unilaterally suprarenalectomized, ovariectomized and hysterectomized during a period of eight days. All of these animals survived, the operated ones showing a drop of 3 to 11 grams after each operation, but returning to the pre-operative weight level on the second or third day.

2. *The effect on the oestrous cycle.* The removal of both suprarenals was followed by a definite effect on the oestrous cycle of the rats. Complete cessation occurred in 22, 8 came into one heat followed by an absence of oestrus, and one showed three extended cycles. The two surviving animals of the series showed no great change in their cycles other than an extended interval to the next oestrus in one case. There was, therefore, a distinct disturbance of the cycle in 93.7 per cent of the rats. The operated and unoperated controls all showed normal cycles and from this it appears that the surgical trauma experienced by the experimental animals is a negligible factor as to its effect on the body weight, oestrous cycle or survival.

C. *The effect of cortico-adrenal extracts.* 1. *On bilaterally suprarenalectomized rats.* In view of the results found after complete suprarenalectomy an attempt was made to determine the efficacy of cortico-adrenal extracts to maintain the life and restore the body weight and normal activity of the ovary. Such an investigation would also determine whether our suprarenal cortical extracts contain an ovary inhibiting substance or possess a sex specific stimulating principle. Bilateral suprarenalectomy was performed at a "one stage" operation on a series of 60 adult virgin female rats four to seven months old after a preliminary period during which each animal showed at least three normal oestrous cycles and a normal increase in weight. The three day pre-operative weight average ranged from 162 to 247 grams. Six rats of this series were operated upon by rigid surgical aseptic technique with results in no way differing from rats operated upon by the ordinary antiseptic method.

Following the removal of both glands, 14 animals showing unmistakable signs of glandular insufficiency with complete cessation of the oestrous cycle for a period ranging from seven to thirty-five days were injected subcutaneously twice daily with extracts of whole suprarenal glands. The extracts were prepared from beef and horse suprarenals according to Pfiffner and Swingle's long (17a) and "one half" methods (17b). In the course of injection therapy, thirteen different batches[2] of extracts were employed. It was found necessary to inject from 1 to 3 cc. per day, which was equiva-

[2] We wish to thank Dr. David Klein of the Wilson Laboratories and Dr. A. E. Meyer of the Chappel Foundation for their generous supply of beef and horse adrenals.

lent to 30 to 120 grams of whole gland. Extracts prepared by the method of Kutz (12) did not maintain the lives of the operated animals even when large amounts were injected.

All injected females showed a return of normal oestrous rhythm for one to five cycles after treatment for 4 to 21 days (table 2). Significantly, however, this occurred only after the body weights had been restored to approximately pre-operative level. Oestrus in all cases but two was

TABLE 2

The effect of cortico-adrenal extracts on the body weight and oestrous cycle of bilaterally suprarenalectomized rats

All were virgin females four to seven months old. One to 3 cc. equivalent to 30 to 120 grams whole gland were injected daily.

	TOTALS AND AVERAGES	REMARKS
Number of rats	14	
Pre-operative weight based on average of three days	162 to 247 grams	
Per cent weight loss	12.2 to 28% Av. 18.5%	2 were prostrate, 2 semi-prostrate
Number of days out of oestrus before injection	7 to 35 days. Av. 10.5 days	
Number of days injected before return of weight to normal at first oestrus	4 to 21 days. Av. 10.6 days	
Per cent of weight lost regained by time of first oestrus	42.5 to 148.% Av. 91%	Weight continued to increase when injections continued
Number of oestrous cycles following the start of injections	1 to 5	
Number of days survival after injections were stopped	5 to 34 days. Av. 11.7 days	2 survived, but showed temporary loss in weight after injections stopped. Cortical material found
Number mated	3	Litter of 4 borne by one; other two aborted after injections stopped on 9th and 16th days

normal and not continuous as would be the case if theelin or gonad stimulating principle were injected. In the two exceptions an atypical smear was found when the animals had regained 71 per cent and 76 per cent respectively of the weight lost. Six rats of the group came into oestrus when the restored body weight was higher than pre-operative average; the others exhibited normal oestrus when close to or slightly below it. In all cases, there was a marked and definite increase in weight after or starting within two days following the beginning of injections.

Some of the experimental females have been kept alive and in apparently normal physiological condition by injection therapy for as long as 70 days, and when injections were stopped death followed in 9 to 14 days. In two cases, death did not follow after the injection was stopped. It was found at post mortem that both possessed cortical rests. Rats in prostrate condition required intraperitoneal injections of 1 to 3 cc. of extract every 3 to 4 hours for recovery. Injections of physiological saline, 1:1000 epinephrine or 10 per cent glucose showed only slight and temporary ameliorative effects on such animals. A few of the rats injected with physiologically active cortico-adrenal extracts during the last twelve or fifteen hours of life were not even temporarily revived from comatose condition. On the other hand, much smaller injections of the same extracts were maintaining the lives of other suprarenalectomized rats. It appears, therefore, that some of the rats entered into an irreversible state such as described by Hartman and Winter (10b) from which it was impossible to revive them.

Many of the injected animals continued to lose weight for a day or two after the injections were started and before the gradual increase began. Once the increase started each succeeding day's weight was characteristically higher than that of the previous day until the weight approached the pre-operative level or somewhat higher. Then, as in the normal unoperated rats, the weight fluctuated from day to day, but with a general trend upward. Rat 12 showed the most rapid increase in weight that occurred in this series. This animal's pre-operative average was 247 grams, and eight days later and the first day that it was injected the weight was 205 grams. The following day it was 200 grams. An abrupt increase to 216, 222 and 231 grams followed during the next three days. On the last day the vaginal smear showed nucleated and cornified cells. The injections were discontinued and the rat died showing typical signs of insufficiency eight days later when its weight was 190 grams.

To demonstrate further that the rats receiving injections had been restored to a normal condition three of the injected females were successfully mated. The first one showed five normal oestrous cycles up to the time of mating and injections were continued to the nineteenth day of pregnancy. A litter of four apparently normal rats was born on the twenty-second day and the mother died six days later showing signs of cortical insufficiency. The second injected female showed four normal cycles before it was mated. In this case the extract was discontinued on the sixteenth day after sperm were seen in the vaginal smear. Abortion occurred eight days later and the animal died on the eleventh day after the injections were stopped. The third female also showed several cycles before it was mated and aborted on the thirteenth day of pregnancy, four days after the injections were stopped.

2. *Extract control experiments on normal animals.* The extracts used in

this work were assayed on dogs following the assay method developed by Harrop, Pfiffner, Weinstein and Swingle (9). Two dogs were completely suprarenalectomized by a two-stage removal with the surgical assistance of Dr. W. J. Meek. It was found that 0.1 to 0.2 cc. of the extract, equivalent to 3 to 6 grams of whole beef adrenal, constituted one dog unit; the relative potency of extracts of horse adrenal glands is only slightly less.

In order to determine if these extracts, which were being injected into some of the suprarenalectomized rats, contained a gonad stimulating principle which might have been responsible for the return of oestrus, the following experiments were performed. First, a series of 21 twenty-two day old immature female rats was used. Of these 14 were injected for three to five days with 2.5 to 10 dog units of the extract per day. They were sacrificed on the sixth day along with the seven uninjected controls and in each case the combined weight of the ovaries recorded. The ovarian weights of each control uninjected rat ranged from 7 to 12 mgm., the average being 9.6 mgm. The ovaries of the injected series ranged from 7.4 to 13.4 mgm., with an average of 10.4 mgm. These weights are within the normal limits.

Second, the Friedman test (7) for gonad stimulating principle was also used. Cortico-adrenal extracts were injected in amounts of five and ten dog units, respectively, into the ear veins of two adult isolated female rabbits. Both rabbits had been previously laparotomized to ascertain that the ovaries were in the "heat" condition. Forty-eight hours after the injections were made laparotomy was again performed showing no indications of ovulation in either case. Five days after the original injections were made, 30 cc. of human urine of pregnancy, which had been shown to be positive for gonad stimulating principle on normal rats and rabbits, were injected into one of the two rabbits and definite signs of ovulation were apparent two days later. This showed that the rabbit could be made to ovulate. The results obtained on the immature rats and on the rabbits indicate that the extracts employed did not contain an ovary specific stimulating principle.

Discussion. The principal factors involved in the disagreement over the survival of rats following suprarenalectomy have concerned the presence of accessory cortical material, the operative skill, the type of technique and the post-operative care of the animals. All the workers in the field agree to the occurrence of accessory cortical tissue, but the reports vary considerably as to its frequency. An apparently important point previously overlooked in this regard has been brought out by Gaunt (8a) whose investigation shows that there may be a colony difference in the survival of rats apparently due to the occurrence of accessory material. The results reported here seem to indicate that accessory tissue was relatively infrequently encountered in these animals. This was demonstrated

by the fact that over 93 per cent died from cortical insufficiency following complete suprarenalectomy.[3] Many investigators (6) (14a) (20) have already pointed out that the exceedingly long survival periods reported by the earlier workers were due either to the presence of accessory tissue or to failure at the operation to remove all of the gland with its pedicle and surrounding connective tissue. Both of these criticisms appear to be very important since it has been shown by Pencharz, Olmsted and Giragossintz (16) and Wyman (20) that small amounts of cortical tissue may hypertrophy and be sufficient to maintain the life of the animal. In operations in this work the pedicle and surrounding tissue were removed and in most cases saved so that a check could be made to see if the capsule was intact in the instances of survival. Unilateral suprarenalectomy and other control operations showed that surgical trauma was a negligible factor in our work. Likewise, our operative procedure with aseptic or antiseptic technique did not alter the results in our studies.

No extensive work has been reported on the body weight changes following suprarenalectomy in the rat, nor has there been a careful comparison made between the survival and body weight loss following "one and two stage" removal of the glands. Gaunt (8a) reported a 15 to 20 per cent loss in weight in rats operated on by the "one stage" method, but did not say how many animals were involved in his calculations, though another section of his report involved over a hundred rats. In the present work two series of 35 and 33 rats each were used and it was found that the percentage loss in weight varied from 13 to 35 per cent, the average being 19 per cent.

A comparison of the survival following "one and two stage" removal of the suprarenals reveals that it is, on the average, three days longer when the two stage method is used. This result explains the difference between the report previously made (14a) of an average survival of 15 days following a two stage operation and those of other recent investigators who obtained averages of 6 to 12 days following a one stage removal of the glands. The body weight loss of animals in the two series is similarly different, though, as with the survival period, the range of weight loss in the two groups is about the same. There was an average weight loss difference of 4 per cent, that of the one stage series being 17 per cent while the average of two stage rats was 21 per cent. No correlation was found between the per cent weight loss in either or the combined data of the two series and the survival period; likewise there was no correlation between the body weight loss and survival period when compared to the pre-operative level.

There now appears to be no question but that the oestrual cycle is definitely disturbed in bilaterally suprarenalectomized rats. The work pre-

[3] In a personal communication, Doctor Gaunt reported that rats obtained from our colony were no exception to the fatal effects of bilateral suprarenalectomy.

sented here agrees with the recent investigations of Kitagawa (11), Martin (14a), Gaunt (8a), Firor and Grollman (5) and Corey and Britton (3b) that the oestrous cycle may be lengthened, irregular, or completely inhibited. Furthermore, it seems, as Wyman (20) has shown, that the degree of suprarenal insufficiency is the governing factor in inducing a hypofunction of the ovary.

Relatively few workers have studied the effects of cortico-adrenal extracts on the oestrous cycle of normal or suprarenalectomized rats. Connor (2), using normal animals, found an inhibition of the cycle when he used a crude saline extract of the glands. On the other hand he reported that a preparation made according to the method of Swingle and Pfiffner had no observable effect on the cycle. Very recently, however, Corey and Britton (3b) stated that "cortico-adrenal extract when injected into normal rats produced a modification of the oestrous cycle in the direction of increased activity, the oestrous type of smear being often observed for several days continuously." These workers did not give any data as to the amount of extract injected. They also found that they were able to restore the inhibited oestrual cycles of suprarenalectomized females by injection therapy. However, no mention was made as to the periodicity of these cycles, the correlated changes in body weight and the length of time beginning the initial injection and appearance of the first oestrus. Our operated rats did not regain their normal oestrous cycles until the body weight had been restored to approximately normal pre-operative level. Also, the subsequent cycles were not continuous but occurred at the normal intervals. Thus the extracts prepared and used in this work have no inhibitory or toxic influence upon the normal cyclic activity of the ovary. It is firmly believed that the appearance of a normal and rhythmic oestrus dependent upon the restoration of the body weight to within normal limits following extract therapy strongly suggests again that the cortical hormone is a general tissue hormone (10c) (14a) (19). Unless the normal physiological condition of the animal is restored, we may expect disturbances of various kinds.

Several investigators have used immature rats in their experiments with cortico-adrenal extracts. Gaunt and Parkins (8b) injected as much as 20 dog units of Pfiffner and Swingle's extract into immature rats, but were unable to demonstrate any effect on the ovary, uterus or vagina. Morrell (15) was unable to produce precocious spermatogenesis in the immature male rat. However, Corey and Britton (3a), who first tried this procedure, reported that they were able to produce precocious sexual maturity in the immature animals, and that the reaction was more marked in the females than in the males. Our results on immature rats and in addition the negative Friedman reaction in female rabbits conclusively show, in our opinion, that the cortical adrenal extracts prepared by the Swingle and Pfiffner method contains no specific ovary stimulating principle.

SUMMARY

Complete suprarenalectomy in 68 female albino rats resulted in the death of 93.9 per cent of the group. The survival period ranged from 6 to 29 days. Definite accessory cortical tissue was found in those animals that survived for extended periods.

Removal of both suprarenals at one operation shortened the survival period. This was three days less than the average of 14.9 days for rats whose suprarenals were removed in two stages.

Following complete suprarenalectomy there was a gradual decline in body weight which at the death of the animal ranged from 13 to 35 per cent. The average weight loss of a two stage operated series was 21 per cent as compared to 17 per cent for a one stage series.

Definite oestrous disturbance occurred in 92.5 per cent of the animals following removal of both suprarenals.

No correlation was found between the degree of body weight loss, the survival period or pre-operative weight of suprarenalectomized female rats.

Potent and standardized extracts of beef and horse suprarenals prepared according to the Swingle-Pfiffner method have successfully maintained the life and restored the normal oestrous cycle of suprarenalectomized rats. The cycles did not reappear until the body weight returned to approximately the pre-operative level. Our results favor the theory that the active principle from the suprarenal cortex is a general tissue hormone.

It is concluded that the extracts employed in this work contained no ovary inhibiting or specific sex stimulating factor.

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OBSERVATIONS ON THE NERVOUS CONTROL OF THE ILEO-CAECAL SPHINCTER AND ON INTESTINAL MOVEMENTS IN AN UNANESTHETIZED HUMAN SUBJECT

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An opportunity was afforded to make observations on the proximal colon, ileo-caecal sphincter and distal ileum in an unanesthetized human subject, a colored woman, aged 30. About a year previously a caecostomy had been performed following a severe colitis involving the transverse and distal colon. Prolapse had occurred, so that the caecum and several inches of proximal colon were everted. The prolapsed colonic tube was double-walled, with the peritoneal surfaces in apposition, the mucosa of the proximal or outgoing segment to the outside and the mucosa of the distal or returning segment lining the tube. The colonic tube lay on the abdominal wall to the right of the wound, the everted caecum lay to the left, and the ileo-caecal orifice was plainly seen (fig. 1). All records published in this paper were made in the morning with breakfast omitted.

A definite reddening of and increased secretion by the mucosa of the proximal colon was always seen immediately on beginning eating. The following notes on one observation are typical. "Before eating the colon mucosa was pale salmon pink and relatively dry, quite sticky. About the time of beginning to eat the mucosa became a definitely deeper red. Within 5 to 10 minutes the color was almost that of tomato catsup and the surface was covered with a thin secretion, was definitely wetter than at first. Observations were continued for 20 minutes after the meal was finished; at that time the depth of color was somewhat less than at the maximum but it was still definitely redder than before the meal." Such a reflex vasodilatation extending to the lower alimentary tract immediately on eating seems surprising, but it was consistently observed.

Movements of proximal colon. These were of three types, first, feeble and completely irregular local contractions reminding one of the movements of a fibrillating cardiac chamber. These might course up or down for a few millimeters but were never transmitted in such a way as to be capable of transporting any material. The extent of this activity was quite variable. It seems improbable that these movements would be detected on x-ray examination. Second, an abortive type of haustral

segmentation was observed. What appeared to be the beginnings of constrictions encircling the colon occasionally appeared at intervals of a few centimeters, but these never became well developed and invariably soon passed off. It must be remembered that this colon is always empty. Third, a very powerful contraction wave, usually beginning at the caecum and passing slowly (a few millimeters per second) along until lost from view. This exerts great force; it is apparently accompanied by a strong contraction of the longitudinal muscle, as evidenced by the definite shortening. This is probably the mass movement described by various observers as occurring occasionally and transferring material to the distal colon or even going on to defecation. This movement only rarely occurred spontaneously and then usually as a sequel to an unusually vigorous peristalsis in the lower ileum, such a wave occasionally passing the sphincter to appear

Fig. 1. Photograph showing caecum, sphincter region and proximal colon

in the colon as the effect described. This powerful contraction of the proximal colon could, however, early in a given period of observation, be elicited by mechanical stimulation (insertion of finger) of either ileum or proximal colon. After several such responses, however, this stimulus was no longer effective, but insertion of two fingers with forcible stretching remained effective after many repetitions. On a few occasions it seemed that a colonic wave starting from the caecum was met several inches below the caecum by an antiperistaltic wave, but the picture was so confused that several observers could not agree on this point. It cannot be said that a clear-cut retrograde movement in the proximal colon was seen. The movements of the colonic wall due to arterial pulsations were surprisingly large and might be a significant factor in changing the colonic contents in contact with the surface. Occasionally but not invariably the

passage of ileac contents through the orifice was preceded by definite relaxation of the proximal colon, presumably the same phenomenon described by Lyman (1913).

Apearance of ileo-caecal sphincter. The sphincter region in our subject corresponded to the description given by Short (1919) rather than that of Rutherford (1914). However, in the fasting state, i.e., 12 to 15 hours after the last food, the passage of the ileac contents through the orifice was a much more frequent occurrence than with Short's subject. In his case such passage was seen only after food or water by mouth; in our subject a definite increase was seen on about half the occasions within a half hour after a meal. The sphincter was relaxed a large part of the time; it was observed many times, both after 12 to 15 hours of fasting and immediately or soon after eating. While, on the whole, the average state of closure is perhaps greater after eating than on fasting, the finding of Hannes (1920) on the dog that the sphincter is always open on fasting and always closed after eating was not confirmed on our subject.

When contracted the sphincter could always be dilated to admit the index finger without discomfort. In view of Rutherford's description of the scarlet-red mucosa of the region and the pain on attempting to insert even a catheter it seems probable that some inflammatory process existed in his case. In our subject the ring of muscle could be felt extending about 2 centimeters when it was squeezed down on the inserted finger; when relaxed the limits of the ring could not be recognized. When the sphincter is contracted it does not invariably relax completely in response to a spontaneous persistalsis reaching it from the ileum. This is evidenced by the fact that at first gas and bubbles may be heard and seen passing through the still puckered lips of the orifice (the region being elevated above the surrounding mucosa when in the contracted state); activity may cease here or additional waves may arrive, in which case the sphincter usually relaxes and ileac contents escape. After such relaxation it may stay relaxed for some time, or several rhythmic contractions may occur after the passage of the wave. It is recognized that the sphincter in a subject with a normally filled proximal colon may be closed a larger proportion of the time than in our subject.

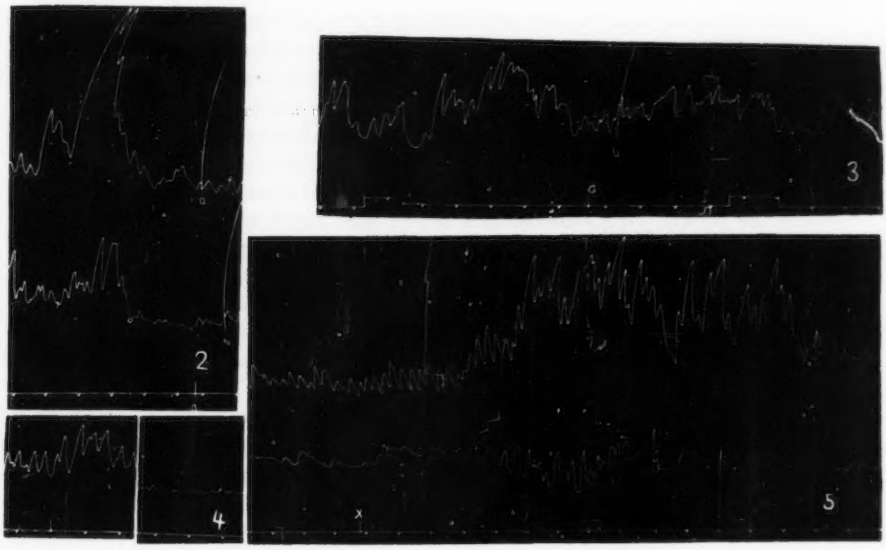
Movements of ileum and sphincter. Records from a balloon[1] in the ileum usually show rhythmic non-propulsive contractions, from 6 to 12 per minute. Whether these were present before the balloon was inserted is

[1] The balloons were of condom tubing tied over glass tubes perforated along the sides and closed distally. The sphincter balloon was constantly held in place by one of us to avoid the possibility that it might be influenced by movements of the ileum. Balloons and communicating rubber and glass tubing were filled with water at a pressure of 12 to 15 cm.; the writing levers were on loose condom rubber tambours, air tight and slightly inflated, which acted practically isotonically within the usual limits of the rhythmic contractions.

not certain, although this type of movement is probably what Short saw through the thin wall of caecum covering folds of ileum, which he described as "incessant peristalsis." True peristaltic waves occurring spontaneously were usually infrequent, perhaps an average of 3 or 4 an hour. At times, usually early in an experimental period, peristaltic waves were seen much more frequently. Our reason for calling these peristalsis is that they produced active expulsion of ileac contents through the ileo-caecal orifice. These contraction waves were not preceded by a wave of inhibition; rhythmic contractions continued with undisturbed rhythm and with little or no diminution in amplitude during the passage of these waves. If these are not true peristaltic waves they at least perform the same function. The interval between the arrival of the foot of a wave at the upper end of a balloon and the departure of the tail of the wave from the lower end (balloon length 5 cm.) is from 1 to 2 minutes. We cannot measure the length of the wave, so can only say that it travels at least 5 centimeters in from 1 to 2 minutes.

Three types of sphincter response, with the sphincter previously relaxed, may be recognized following a peristaltic wave in the ileum. First, the sphincter may contract within a few seconds of the beginning of the peristalsis (fig. 2). It seems improbable that a propagated wave could have reached the sphincter from the upper end of the ileum balloon (7 to 8 cm. from the sphincter) within this interval. This therefore appears to be a reflex contraction of the sphincter in response to an approaching wave in the ileum. Second, the sphincter may contract at from 30 to 90 seconds after a wave reaches the upper end of the ileum balloon. These responses are probably a continuously propagated wave of contraction passing down to the sphincter, although they may be mere coincidence; the latter does not seem probable. Third, the sphincter may show no response to peristaltic activity in the ileum. In no case does the sphincter respond to activity of the ileum with a maximal contraction. The latter is seen in response to a powerful contraction of the proximal colon. Records of this cannot be shown, since such a colon contraction interferes with the record from the sphincter, but that the sphincter was much more powerfully contracted than following peristalsis of the ileum was evident from inspection. During contractions of the type shown in figure 2, the sphincter region is only slightly elevated, the ring feels somewhat firmer than the surrounding tissues and the contraction is obviously not maximal. The contractions that accompany the third type of colon activity of the second paragraph show the sphincter region considerably elevated, protruding like a firm nipple, the orifice is slit-like, the ring is of almost cartilaginous consistency and a finger can be inserted only with difficulty. This contraction will persist unabated for one or more minutes. The sphincter response to an ileac wave reaching the ileo-caecal junction is, on the other hand, typi-

cally a series of rhythmic contractions. Hertz (Hurst) (1913) describes the sphincter as relaxing with each peristaltic wave reaching the terminal ileum. At first thought this description as well as our observation of rhythmic activity seems inconsistent with Hertz' view that the sphincter is largely responsible for the delay in passage of material from ileum into caecum, as seen in man. The crucial point is the extent to which the



Figs. 2–5

Fig. 2. Spontaneous peristalsis resulting in expulsion of ileac contents. In all figures where both records are shown the ileum record is above. Time record at bottom shows 30 second intervals in all records when present.

Fig. 3. Record of sphincter alone. During elevated segment of time record balloon in ileum was distended by 30 cm. of water. Sphincter responded to first distention by contracting, no response or possibly inhibition to second.

Fig. 4. Sphincter alone. At signal on time record 0.3 mgm. adrenalin subcutaneously. Second part ten minutes later.

Fig. 5. Seven one hundredths grain pilocarpin hydrochloride subcutaneously at *X*.

sphincter is normally closed. If it is usually closed it would retard passage even though it opens to each wave which reaches it, for many of the waves of the distal ileum fail to reach the sphincter. This latter statement can be proved by inserting a finger through the sphincter as far as possible into the ileum; waves can be felt coming up to the tip of the finger and dying out before reaching the base. On the other hand, if the sphincter is usually

relaxed, the delay must be ascribed to the paucity of peristaltic waves in the terminal ileum, the physiological stasis discussed by Hertz.

The question remains as to the factors which determine the state of closure of the sphincter. If the presence of material in, i.e., distention of, the terminal ileum is responsible for closure, this factor should be normally operative in our subject. That the passage of material from the ileum was delayed even more than is normally the case is shown by the fact that the material escaping was usually semi-solid and at times almost as dehydrated as normal faeces; nevertheless the sphincter was relaxed more than half the time, even after eating. If, on the other hand, distention of the proximal colon is mainly responsible for closure, it is not surprising that the sphincter should be so relaxed in our subject. As stated above, it was obvious that the contraction on distention of the proximal colon was much more forcible than that occurring spontaneously or after a peristaltic wave from the ileum. We confirm Hinrichsen and Ivy's observation (1931) that distention of the distal ileum may or may not be followed by a sphincter contraction (fig. 3). The impression is thus given that the responses of the sphincter are such as to be more effective in preventing retrograde passage from the colon than in retarding passage from the ileum; this impression is not necessarily correct if the sphincter is normally closed more than was the case in our subject.

The innervation of the sphincter. The sympathetics are described as the motor supply to the sphincter in the dog, cat and rabbit, the parasympathetics as being without effect (Elliott, 1904; Dale, 1906, Kuroda, 1916). Katz and Winkler (1903), however, found that in the dog vagus stimulation usually closes the sphincter and splanchnic opens it; Elliott questions the validity of their technique. Hinrichsen and Ivy confirm on the dog the finding that splanchnic stimulation and adrenalin have a pure motor effect on the sphincter but find that the vagus also is effective, producing an initial relaxation followed by contraction. We have recorded the response of ileum and sphincter to subcutaneous doses of adrenalin. With the balloon in the sphincter there is often a rhythmic activity; the period bears no relation to that of the ileum, usually being slower but sometimes faster. Much of the time the sphincter is relaxed and shows no activity at all while the ileum rhythm continues. In seven experiments it was shown beyond question that in from 5 to 15 minutes (usually 7 or 8) after the subcutaneous injection of 0.3 to 0.6 mgm. of adrenalin chloride followed by massage of the site of the injection, there was a progressive diminution in rhythmic activity and fall in tone of the ileum accompanied by a cessation of rhythmic activity (if such had existed) and loss of tone (if such had existed) of the sphincter. If the sphincter was quiet and relaxed before adrenalin it remained quiet, and relaxation became even more complete (fig. 4). A similar result was obtained in one experiment on the local

application of several drops of 1/1000 adrenalin to the sphincter region. Moderate blanching of the mucosa was seen with this application; at lower concentrations no definite blanching and no effect on muscular activity were seen. In no case was a motor response to adrenalin seen. The subcutaneous injection of 0.5 cc. of 0.9 per cent NaCl solution was without effect. It thus appears that in the human subject, contrary to reports on lower animals, adrenalin inhibits rather than stimulates sphincter activity. In addition to the species difference in subjects, the two most obvious differences between our methods and those of earlier workers are 1, that we used an unanesthetized subject, and 2, we used smaller doses of adrenalin. It may be that the relatively brief sphincter spasm seen in animals with large doses intravenously is not a normal response to more nearly physiological concentrations, such as we employed. Hinrichsen and Ivy used smaller doses than did Elliott but even their dose (0.1 cc. of 1/10,000 intravenously for a cat) undoubtedly produced temporarily a considerably higher concentration of adrenalin than did our doses.

Our subject showed definite nervousness with 0.3 mgm. subcutaneously. This reaction began from 4 to 10 minutes after administration; at about this time a moderate rise of blood pressure (averaging about 20 mm. systolic and 15 mm. diastolic) began, as well as a marked rise in pulse rate (15 to 50 per minute). Inhibition of intestinal activity usually was first manifested only after the above changes had begun; the inhibition lasted for many minutes.

No definite change in either ileum or sphincter was seen after 0.05 grain of pilocarpin hydrochloride subcutaneously. Both were showing rhythmic activity before; this was perhaps somewhat greater afterwards but the variation was not outside the spontaneous fluctuations often seen. No more definite results were obtained with pilocarpin applied locally to the sphincter region. After 0.07 grain subcutaneously, however, there were an increased tone and rhythmic activity of the ileum and increased rhythmic activity of the sphincter (fig. 5).

A variety of intestinal responses to pituitrin has been reported by many observers; see Gruber and Robinson (1929). On four occasions 1 cc. "surgical" pituitrin was given subcutaneously to our subject, followed by massage. On three of these there was great diminution in tone of the ileum and almost complete cessation of rhythmic activity, the effect being first observed in about five minutes, reaching a maximum in about ten minutes and persisting for forty to fifty minutes. Peristaltic activity in the ileum was definitely less frequent after the injection in these three cases. On the fourth occasion there was also a diminution in tone of the ileum and some diminution in rhythmic activity but peristalsis was considerably increased in force and frequency. In every case there was some increase in frequency of the powerful colon contractions; this increase might be barely

perceptible in one experiment and definite in the next. The distal colon seemed to be more sensitive than the proximal; the subject might complain of cramps in the left abdomen when no movement of the proximal colon was taking place. At other times a contraction of the proximal colon apparently passed all the way, as the subject would complain of cramps in the left abdomen within a few seconds of the beginning of a contraction in the proximal colon, and might pass a slight amount of mucus by rectum. There was usually some increase in rhythmic activity of the sphincter following pituitrin; this might persist even with complete absence of activity in the ileum. Our findings agree approximately with those of Carlson (1930) on the human colon but our ileum responses were less uniform than his. In view of the consistent increase in colon activity and the occasional increase in ileac peristalsis, the therapeutic use of pituitrin for intestinal distention in the human subject seems indicated, although the diminution of tone and rhythmic activity and the not infrequent lessening of peristalsis in the ileum point to the desirability of a much larger series of observations on the human subject.

SUMMARY

Observations and graphic records of the proximal colon, ileo-caecal sphincter and terminal ileum were made on an unanesthetized human subject.

No definite antiperistalsis was seen in the proximal colon.

The ileo-caecal sphincter was relaxed much of the time.

The relaxed sphincter may respond to a peristaltic wave in the distal ileum with an immediate or with a delayed contraction or series of contractions, or it may show no response. The sphincter may show rhythmic activity in the absence of ileac peristalsis.

Rhythmic segmentations of the ileum persist during the passage of a peristaltic wave.

The wave of peristaltic contraction is not preceded by a wave of inhibition.

Many of the peristaltic waves of the terminal ileum die out shortly before reaching the sphincter.

The sphincter is consistently relaxed by adrenalin; its activity may be somewhat increased by pilocarpin.

Pituitrin consistently produces some increase in activity of the colon and a diminution in tone and rhythmic activity of the ileum; peristalsis of the ileum may be either increased or decreased.

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ABSORPTION OF ISOTONIC FLUIDS FROM THE SUBARACHNOID SPACE

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For many years there has been general acceptance of the view that fluids introduced into the subarachnoid space under pressures higher than normal are absorbed. Furthermore, it has been learned that the higher the pressure of introduction of these foreign solutions, the more rapid is the process of absorption. Most of the work on which these generalizations are based was done many years ago (Duret, 3; Spina, 8; Adamkiewicz, 1; Falkenheim and Naunyn, 4; Naunyn and Schreiber, 7; Hill, 6) and the pressures employed were poorly controlled or of an excessive nature. Because of the inadequacies of the data presented in the literature, an attempt was made to investigate the whole problem of the rate of absorption of an isotonic fluid introduced into the subarachnoid space.

METHOD OF INVESTIGATION. A series of dogs, anesthetized with ether and maintained in the horizontal position, was employed under standard conditions of experimentation. The intracranial venous pressure was determined in the superior sagittal sinus (Weed and Hughson, 11). The subarachnoid space was entered by occipito-atlantoid puncture into the cisterna magna and the puncture-needle connected, with as little loss of cerebrospinal fluid as possible, to a three-way stopcock. To the straight arm of the stopcock was attached an open-end manometer of 1 mm. bore, while to the third arm of the stopcock there was connected a special pressure and measuring device (fig. 1, A).

This device for the measurement of intracranial volume-changes by an external system under constant pressures consisted of a graduated pipette connected through rubber tubing to a reservoir of large diameter (6 or 12 cm.). The apparatus was set up as follows: In assembling, the perpendicular pipette was first completely filled with Locke's solution and the reservoir and rubber tubing then filled with a bland oil, colored with Sudan III. The rubber tubing was next attached to the upper end of the pipette with the oil forming an easily visible meniscus on the Locke's solution. Fluid was then withdrawn from the lower end of the pipette to any desired level, the oil following the Locke's solution down the tube. The clear differentiation of the meniscus permitted easy readings of the volume in the

calibrated pipette. By changing the level of the large reservoir in the system, any desired pressure could be maintained in the subarachnoid space as such quantities of fluid as were absorbed by the animal did not appre-

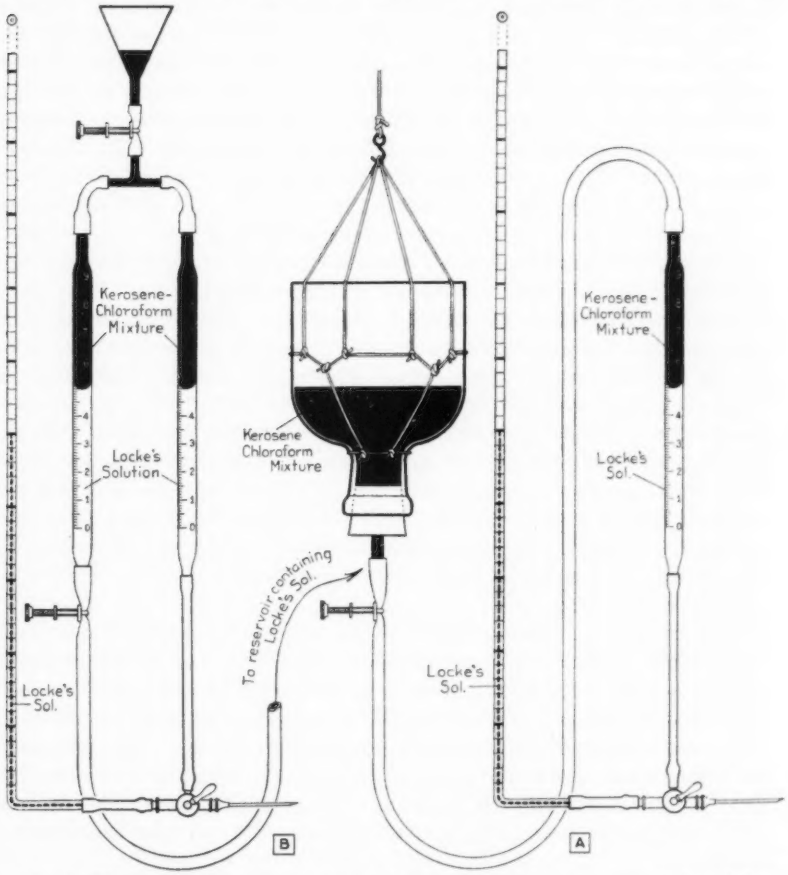

Fig. 1. Diagrammatic representation of reservoir-pipette system employed for measurement of volume-changes under maintained pressures. The clamps shown on the rubber tubing are used during assembly of the apparatus. In the balanced pipette system (B), the clamp on the rubber tubing between funnel and pipette is opened during introduction of the kerosene-chloroform mixture and is then closed during remainder of experiment.

ciably alter the level in the reservoir. It was possible through this means to record the volume of fluid passing from the external system into the animal or that extruded from the animal, under conditions of established

pressure as recorded in the open-end manometer. The only limitation to use of the apparatus relates to the inertia of the fluids; this inertia was not found to interfere at all in its employment in these observations.

In the earlier experiments a bland mineral oil was employed, but because of its viscosity it was replaced by kerosene. Both of these oils had one disadvantage, in that their specific gravity was less than that of the Locke's solution; and with the lowering of the meniscus in the pipette (due to inflow into the subarachnoid space), the subarachnoid pressure as recorded in the open-end manometer fell slightly, particularly at high pressure-levels. This fall was apparently due to the change in relative lengths of the two columns of fluid in the perpendicular pipette. While the lowering of the pressure was of small moment (1–10 mm.), the difficulty was avoided by the addition of chloroform to the oil so that the specific gravity of the mixture became identical with that of Locke's solution.

During periods of rapid change in the extrusion or inflow of fluid the meniscus became irregular because the oil did not retract completely or evenly from the sides of the pipette. This made accurate readings of the volume-changes impossible. This condition was remedied by the use of a balanced system of two vertical pipettes connected by rubber tubing. The upper half of each pipette and the tubing connecting them were filled with the chloroform-kerosene mixture. Under conditions of either extrusion or inflow, one of the two menisci thus provided became easily and accurately readable. In such an arrangement of pipettes the reservoir connected to the second pipette at its lower end is necessarily filled with Locke's solution (fig. 1, B).

In a few of the animals of the series, the subarachnoid space was entered by a double needle, one lumen of which was attached to the reservoir system and the other to the open-end manometer. This double needle was employed to eliminate any possibility that the pressures from the reservoir were communicated only to the manometer through the 3-way stopcock: with the double needle the pressure of the reservoir could be transmitted to the manometer only through the subarachnoid space. The findings with this system were quite similar to those of the simpler experimental set-up.

Experimental findings. With the apparatus attached to the animal as indicated, readings of the normal pressure of the cerebrospinal fluid for a period of some minutes were made. As soon as the pressure of the fluid came to its normal level, as determined by lack of change for 5 to 10 minutes, the reservoir-system was connected by opening the 3-way stopcock, and the level of the reservoir was adjusted so that the normal pressure of the individual animal (as indicated in the open-end manometer) would be maintained.

At normal pressures. With the reservoir set to keep subarachnoid pres-

sure at its normal level, it was quickly apparent that volume-changes of small but easily measurable magnitude occurred in definite cyclic form within the cranium. These volume-changes were usually associated with changes in the pressure of the cerebral venous system, an increase in venous pressure being accompanied by an extrusion of fluid into the external system and a decrease by fluid passing from the pipette into the animal. At times slowly effected venous pressure-changes were not reflected in volume-changes in the external system; and occasionally when a period of extrusion or inflow of fluid was changing into the opposite phase, no change in cerebral venous pressure was noted in the first minute or so of observation. Yet the relation of cerebral venous pressure-change to volume-change in the external system was clear-cut.

Considerable variation in the length of the periods of extrusion or introduction of fluid, as determined by volume-change in the pipette-reservoir system, was noted. Many animals went through periods of 5 to 10 minutes during which fluid would pass from the external system into the subarachnoid space; these periods were followed by intervals of similar time during which extrusion of fluid into the external system took place. In other animals the changes in these phases occurred very much more rapidly, the intervals being of only 1 or 2 minutes' duration. And in these or other animals, there were frequent periods during which no changes in the volume of the external system were recorded. Yet all of the dogs exhibited definite cycles in one form or another, in this process of extrusion or introduction of fluid, when the pressures of the subarachnoid space were maintained at normal levels.

These phenomena of cyclic change are illustrated in figure 2, taken from a dog in which the pressure in the sagittal venous sinus varied considerably from minute to minute throughout the period of observation even though the pressure in the subarachnoid space was maintained at its normal level. The particular record is used as the marked changes in the cerebral venous pressure are accompanied by marked volume-changes in the external system. Periods of extrusion of the fluid are followed by periods during which fluid passes into the animal; at the end of the period portrayed (25 minutes) the total external volume is recorded at exactly the same level as at the beginning of observation.

Such a return of the total external volume to its original reading was obtained only in those animals in which the normal pressure of the cerebrospinal fluid was accurately ascertained. In other dogs, due to error in the establishment of the exact level of normal pressure of the cerebrospinal fluid (particularly in those animals in which wide fluctuations in this pressure were encountered), the setting of the reservoir might be such that a level above or below the normal pressure of the fluid was established. Under these conditions a maintained subarachnoid pressure less than the

normal was shown by a constant but slow extrusion of fluid into the external system, while a maintained subarachnoid pressure greater than the normal exhibited a constant though slow inflow into the animal from the pipette-reservoir system. Some cyclic variations in the inflow or extrusion of fluid were occasionally observed in such animals, but the general

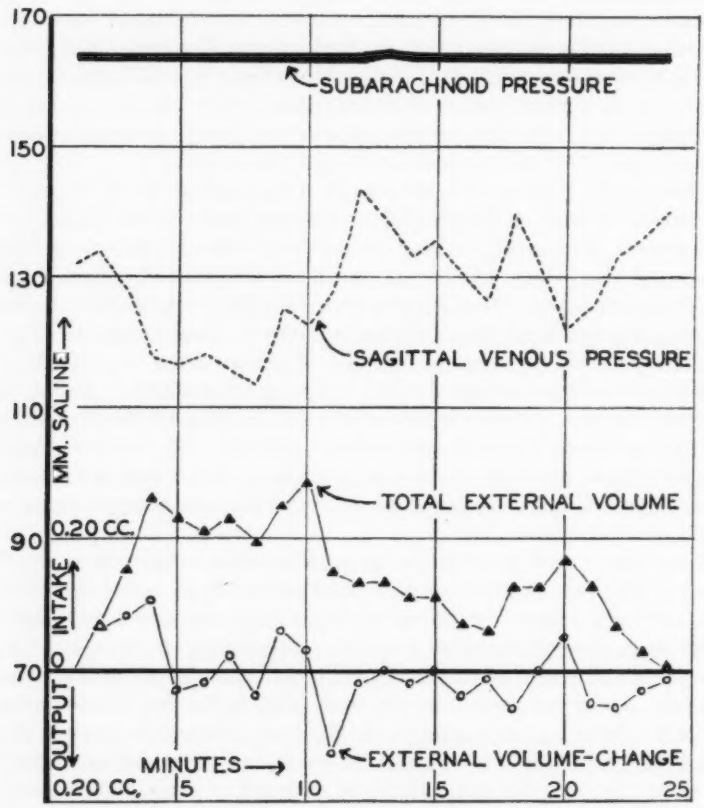

Fig. 2. Dog G 14, with connection of subarachnoid space to reservoir-pipette system, set at normal pressure of the cerebrospinal fluid in this animal. The relationship of sagittal venous pressure to external volume-change and total external volume is shown.

tendency of reaction was clearly indicated by the change in total external volume.

At pressures above normal. As soon as observations at the normal pressures of the cerebrospinal fluid were completed in the individual animal, the

reservoir was elevated; the pressure within the subarachnoid space was thereby raised to any desired height, as determined in the open-end manometer. At all pressures above the normal, inflow of fluid took place—i.e., fluid was introduced into the animal from the external system. In the earlier experiments pressure-increases in steps of 50 to 100 mm. of saline

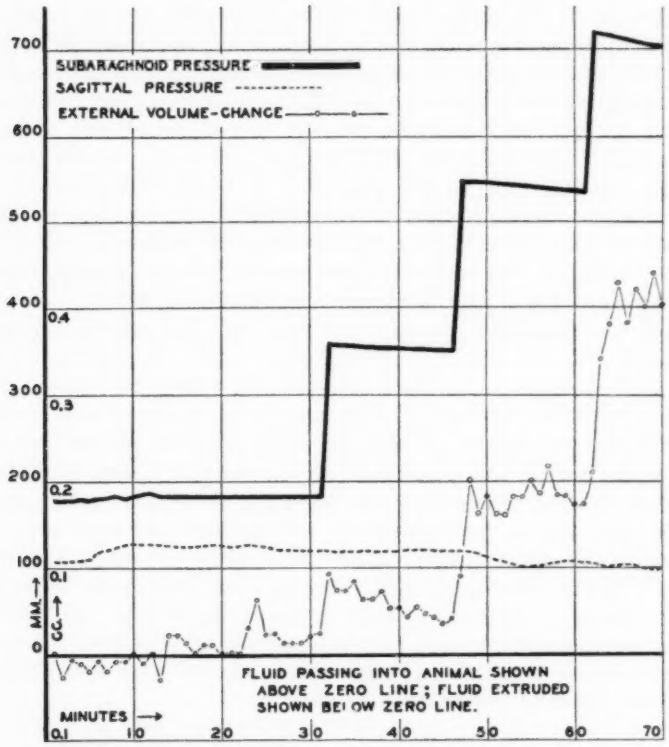

Fig. 3. Dog G 19, with connection of subarachnoid space to reservoir-pipette system, and with measurement of sagittal venous pressure. The initial 30 minutes of the record is at normal pressure of the cerebrospinal fluid, and the remainder at multiples of this normal subarachnoid pressure. The reservoir in this experiment was filled with kerosene and not with the kerosene-chloroform mixture; a slight falling-off of the maintained pressure is indicated.

were carried out. Because of the great variation in normal pressures of the cerebrospinal fluid, it was quickly found that such arbitrary steps in increasing pressure were not as satisfactory as multiples of the original pressure of the cerebrospinal fluid in the individual animal. The later experiments

were therefore all carried out by increasing the pressures in multiples of the original pressure. Comparable data were thus obtained.

The quantity of isotonic fluid required by each animal in raising the pressure in the subarachnoid space to double, triple and quadruple the normal pressure was measured. In the series of dogs, an average of 1.30 cc. of fluid was introduced to double the normal pressure, while an average introduction of 0.83 cc. was required to raise the pressure from 2 to 3 times the normal and 0.94 cc. from 3 to 4 times. Only one dog out of 13 in this particular series required more fluid to pass from 2 to 3 times normal pressure than from 1 to 2 times normal. In the third step (from 3 to 4 times normal), six dogs in the series needed slightly more fluid from the pipette-reservoir system than in the step at lower pressures. In the scattered observations with pressure-elevations from 4, 5 and 6 times normal pressure, smaller amounts of fluid were required at each successive increase. Much individual variation from animal to animal was noted in all of these pressure-increases, the first step (from normal to twice normal pressure) exhibited extremes of fluid introduced of 1.70 cc. and 0.66 cc. There was no apparent correlation between the fluid required and the intradural volume.

Figure 3 gives in graphic form a record of a typical experiment of this nature. When this animal was connected to the pipette-reservoir system at the normal pressure of the cerebrospinal fluid (about 190 mm. of saline), fluid at first passed from the animal into the external system and then the flow was reversed. Soon after the beginning of the record the pressure in the sagittal sinus rose slightly and subsequently continued practically unchanged throughout the rest of the experiment, demonstrating the lack of influence of pressure-change in the subarachnoid space upon pressures in the cerebral venous system (Becht, 2; Weed and Flexner, 10). As soon as the subarachnoid pressure was doubled a definitive inflow of fluid into the animal was recorded, and larger rates of inflow were noted with the higher multiples of the original pressure.

In table 1 the exact quantities of Locke's solution (cc. per min.) introduced into the animal at normal pressures and these multiples of normal pressure are set down in tabular form. Great individual variation is to be noted, even when correction is made for the absolute levels of the pressure of the cerebrospinal fluid. Yet the average rate of absorption as indicated shows a definite increase as the pressure of the subarachnoid space is raised. With the animals graded as accurately as possible in three age-groups, it becomes apparent that there is no relation between the age of the dogs and the exact rate of inflow of fluid from the external system. Furthermore, there is no relation between the rate of inflow of fluid and the weight of the animal, its spinal length and its intradural volume. This individual variation in rate of inflow of fluid, under different pressures, holds when

correction to absolute pressures rather than to multiples of the original pressure is made.

Plotting of the individual rates of inflow of the isotonic fluid (cc. per min.) against pressures (absolute values or multiples of the original pressures) of the cerebrospinal fluid reveals no linear relationship. The curves all show a sharp ascent at the higher multiples, but do not coincide closely.

In many of the dogs it was not possible to increase the subarachnoid pressure to more than 5 times the normal, without causing respiratory and vascular complications. Half of the animals given in table 1 withstood these higher pressures without difficulty: in these the rate of absorption at

TABLE 1

EXPERIMENT NUMBER	SPINAL LENGTH	WEIGHT	INTRADURAL VOLUME	NORMAL C. S. F. PRESSURE	INFLOW 1x NORMAL PRESSURE	INFLOW 2x NORMAL PRESSURE	INFLOW 3x NORMAL PRESSURE	INFLOW 4x NORMAL PRESSURE	AGE-GROUP
	mm.	*gm.*	*cc.*	*mm.*	*cc.*	*cc.*	*cc.*	*cc.*	
G 14	340	4722	59.7	168	0.011	0.071	0.270	0.575	Immature
G 21	410	7210	86.2	136	0.001	0.073	0.171	0.433	Immature
G 24	420	6110	77.2	107	0.000	0.024	0.112	0.173	Immature
G 4	390	7735	72.7	106	0.001	0.013	0.019	0.044	Young adult
G 18	432	7050	82.0	135	−0.004	0.070	0.131	0.310	Young adult
G 25	435	4455	70.8	90	0.001	0.071	0.100	0.156	Young adult
G 27	400	4595	61.4	176	0.001	0.093	0.272	0.735	Young adult
G 28	427	6125	66.2	112	0.005	0.069	0.142	0.236	Young adult
G 19	468	6555	70.8	170	0.000	0.059	0.177	0.353	Adult
G 22	438	7510	70.0	127	0.006	0.060	0.130	0.410	Adult
G 23	455	7500	77.4	104	0.000	0.030	0.079	0.138	Adult
G 26	420	6890	70.3	125	0.001	0.022	0.122	0.246	Adult
G 29	418	6010	62.6	170	0.000	0.046	0.107	0.206	Adult
Average					0.002	0.054	0.139	0.309	

5 times normal pressure was 75 to 100 per cent in excess of the rate at 4 times the normal. Three animals subjected to 6 times normal subarachnoid pressure showed further increases in the rate of inflow of fluid from the external system, roughly 50 per cent over the rate at 5 times normal subarachnoid pressure.

At pressures below normal. Observations of fluid extruded from the subarachnoid space at pressures 50 mm. of saline below the normal level of the cerebrospinal fluid were made on four dogs. In each of these animals a definite extrusion of fluid occurred, an output of 0.017, 0.013 and 0.015 cc. per minute being recorded for three of the dogs. The other animal showed an output of fluid of 0.004 cc. per minute.

Discussion. The findings recorded above add data regarding absolute amounts of isotonic fluid passing into the subarachnoid space of an animal, under conditions of maintained pressure: they confirm the conclusions of older workers that the higher the pressure of introduction of the fluid the more rapid the rate of absorption. For the first time, however, information of a more exact character than that previously available, is given.

In one particular the present findings are of importance in showing that when normal subarachnoid pressures are maintained by an external system, variations in the external volume occur. This finding must necessarily be related to current conceptions of the Monro-Kellie doctrine (cf. Weed, 9) of fixed intracranial contents. Under the conditions of experimentation, with the animal constantly in the horizontal position and with positive pressures constantly present in the subarachnoid space, the dural sac may be taken to be of unchanging capacity. It is apparent that the experimental connection of the pipette-reservoir system to the subarachnoid space destroys the closed-box character of the dural sac: it is equivalent to using the dural sac, inelastic against outward distention, as an oncometer in the experimental system. Such volume-changes as are recorded, under conditions of maintained normal pressures within the subarachnoid space, represent changes which could not have been effected in the intact dural sac. These volume-changes however would seem to have significance in any understanding of the reciprocal relationships which exist between blood, brain and cerebrospinal fluid—the three elements filling the dural sac.

Under the conditions of experimentation, the brain may be assumed to be of unchanging volume,—an assumption which leaves the intradural blood and the cerebrospinal fluid as the only reciprocally varying elements. The actual measurement of volume-change is made from the subarachnoid space which in turn is directly connected to the cerebral ventricles. The volume-changes recorded in the external system represent the changes in the volume of the combined ventriculo-subarachnoid system. They occur in most outspoken fashion when rapid changes in the pressure of the cerebral veins are recorded. Such cerebral venous pressure-changes are customarily reflected in alterations in the pressure of the cerebrospinal fluid (Becht, 2; Weed and Flexner, 10). But to be effective, such pressure-changes in the cerebral venous system must be accompanied by volume-changes in the cerebral veins. With the experimental opening of the dural sac for attachment of the external measuring system, these venous volume-changes can take place without affecting subarachnoid pressure. They are shown as increases or decreases in the ventriculo-subarachnoid volume; and as such may be measured in the pipette system.

The periodic alteration in volume of the ventriculo-subarachnoid system is apparently comparable to the periodic volume-changes of the cerebral

ventricles, as noted by Flexner and Winters (5) in their study of the rate of production of cerebrospinal fluid in cats. These workers observed periods of output of fluid which were followed by periods of no output and even of retrograde passage back into the cerebral ventricles.

In the intact animal such cyclic phenomena must occur, but the physiological result is apparently quite different than in the experimental set-up here recorded. The external measuring system permits changes in the ventriculo-subarachnoid system to occur without change in the total quantity of cerebrospinal fluid, which during the period of observation must be looked upon as fairly constant (cf. Flexner and Winters, 5). But in the intact animal, with no artificial openings into the ventriculo-subarachnoid spaces, such cyclic volume-changes, clearly dependent upon pressure- and volume-changes in the cerebral veins, must be represented by increases and decreases of the total quantity of cerebrospinal fluid. A decrease in quantity of cerebrospinal fluid could only be brought about by increased absorption of the fluid while an increase would follow an increased elaboration of the fluid. This leads then to the idea that there are cycles of active production of the cerebrospinal fluid (as noted by Flexner and Winters) and reciprocal periods of active passage of the fluid back into the blood-stream. This tentative conclusion rests largely upon the assumption of periodic volume-change in the cerebral venous (and capillary) bed and unfortunately no method is at hand to give an accurate determination of this venous volume. The apparatus here employed may however lead to determination of the relative quantities of cerebral venous blood.

SUMMARY

By means of an apparatus capable of maintaining any desired pressure within the subarachnoid space and of recording volume-changes occurring in the space, it has been shown that under conditions of normal pressure periods of extrusion of the fluid from the animal into the external system are followed by periods in which fluid passes from the external system into the animal. Such alternations of flow were usually accompanied by pressure-changes in the cerebral veins, indicating venous volume-changes and consequent reciprocal volume-change in the ventriculo-subarachnoid system. As the pressure in the subarachnoid space was increased an increase in the rate of absorption of fluid was recorded. There was no correspondence between the absolute rate of absorption of fluid, under given pressure-conditions, and the weight, the intradural volume, spinal length and the age of the animal.

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THE RELATION BETWEEN CIRCULATORY RATE AND ABSORPTION IN THE GUT

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During the past few years several hundred experiments on the absorption of glucose from the gut of the frog were performed in order to study the effect of various hormones and ions on this process. In these experiments the perfusion rate of Ringer's solution in the blood vessels supplying the gut was kept as constant as possible since it was our objective to study the effect of various reagents on the permeability of the gut. But not only the membrane characteristics are of importance to the absorption process but also the conditions of the circulation through the blood vessels. It is conceivable that alterations in the total circulatory flow through the intestinal blood vessels might bring into play several opposing factors. Since the membrane characteristics remain constant if the preparation is perfused with oxygenated Ringer's solution the absorption rate must be dependent on two factors: 1, the area across which absorption occurs, and 2, the rate of flow in the individual capillaries. Both factors are obviously interrelated since an increase in perfusion pressure might not only alter the absorbing area by opening up closed capillaries but also change the rate of flow in individual capillaries. A systematic study of changes in the total circulatory flow in the gut vessels on the absorption from the gut seemed to us of interest for the absorption problem as such as well as a means of studying the capillary circulation in the gut during the absorption process.

The method (perfusion of the gut of the frog wih isotonic glucose and perfusion of the blood vessels supplying the gut with Ringer's) was the same as in our previous papers which may be consulted for details (Gellhorn, Northup and Skupa, 1933).

RESULTS. The effect of variations in perfusion pressure which lead to corresponding changes in the total flow of Ringer's per unit of time is not the same in all preparations. It may, however, be adequately described by dividing the experiments into several groups. In the first group it was found that within very wide limits *the concentration of glucose in Ringer's resulting from the absorption from the gut remained constant in spite of considerable changes in the circulatory rate.* In figure 1 a typical experiment is reproduced. In this figure, as well as in the following figures, the concen-

tration of glucose, the amount of Ringer's which circulates through the gut in a 10-minute period and the total amount of glucose absorbed from the gut in this time are plotted in percentages of the value obtained from the first perfusion period. It is apparent that the concentration of glucose shows very slight variations except that it gradually decreases in two hours by 13 per cent of its original value. This slight decline is independent of variations in the perfusion rate and was frequently observed in our experiments. It was already discussed in the previous papers of this series as indicating a slight decrease in the gut permeability. In the periods 3, 4, 8,

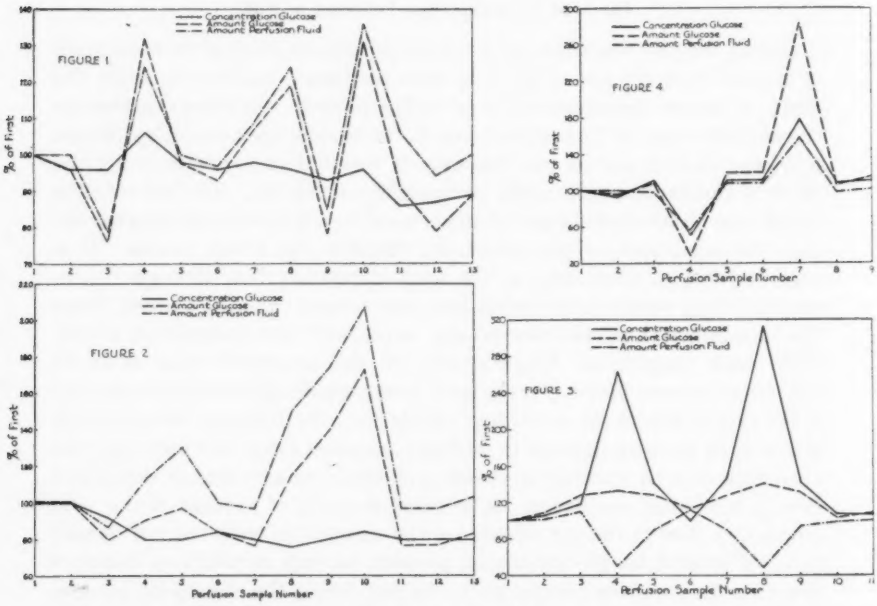

Fig. 1–4. In all figures the values for the various variables are given in percentage of those obtained during the first 10-minute period.

9 and 10 the perfusion rate was either increased or decreased for 20 to 35 per cent. As a result of the remarkable constancy of the glucose concentration it is found that the curve representing the total amount of glucose absorbed in each period is extremely close to the curve representing the perfusion rate through the gut. Still more striking is the experiment reproduced in figure 2. From the 4th to 13th period the glucose concentration is almost exactly 80 per cent of the amount first absorbed and maintains this value in spite of the fact that the perfusion rate was increased by more than 100 per cent. In some preparations the circulation rate could be tripled,

or decreased by 60 per cent, without appreciably altering the concentration of glucose in the perfusate. In all, seven experiments were obtained in which alteration in rate of perfusion led to no change in the concentration of glucose in the perfusate.

In another group of experiments exactly the opposite reaction was observed: The concentration varied inversely as the perfusion rate; the amount absorbed per unit time remained constant, in spite of wide variations in the amount of fluid passing through the capillaries. There were four experiments in this group; figure 3 is an example.

Still other experiments were of an intermediate type. When the perfusion rate was increased, the concentration of glucose in the perfusate decreased, but not enough so that the amount absorbed per unit time remained constant, in fact it rose. A decrease in perfusion rate led to the expected inverse results. There were twelve preparations which gave results of this type.

TABLE 1

PERFUSION PRESSURE IN CM. H_2O	TOTAL PERFUSION IN 10 MINUTE PERIOD		PERFUSION PRESSURE IN CM. H_2O	TOTAL PERFUSION IN 10 MINUTE PERIOD	
	Absolute	Relative		Absolute	Relative
	cc.	*per cent*		*cc.*	*per cent*
14–15	4.4	100	13	3.6	100
13	2.4	55	13	3.6	100
15	4.5	102	14	5.2	144
13	2.1	48	12.5	3.8	105
15–16	4.6	104	16	8.0	222
18	9.1	206	12.5	3.7	103

In addition, about half the experiments performed gave results which showed a composite type of response. The same alteration of the perfusion rate would produce first a reaction corresponding to one of the groups described above and then at a later time the reaction of another group. In one preparation a very striking result was obtained. A decrease in the perfusion rate led to a *decrease* in the concentration of glucose in the perfusate, and consequently to a disproportionately large decrease in the amount absorbed; and an increase in the perfusion rate caused an increase in concentration, with, therefore, a profound increase in amount. Figure 4 shows the results graphically. All effects obtained in these experiments were completely reversible.

It may be mentioned that the pressure changes necessary to produce the desired changes in perfusion rate were in all groups relatively small as is exemplified in table 1. There is no relationship whatever between the original perfusion pressure or the pressure variations employed to vary the

perfusion rate and the reactivity of the preparation in regard to glucose absorption.

Discussion. The increase in circulation rate due to an increase in perfusion pressure may alter the relationship between the circulatory and the digestive system in several respects, which may account for the variations in absorption occurring under these circumstances. First it is possible that the permeability of the capillaries is altered. A critical discussion of the problem of capillary permeability (Gellhorn, 1929, pp., 284–301) has shown that variations in the capillary diameter as such do not alter its permeability but variations in capillary pressure may do so (Landis). It is highly probable that this factor is of no importance in regard to our problem since the variations in pressure necessary to produce changes in perfusion rate were in most cases so slight that the alteration in capillary pressure must be negligible. Furthermore, it was found in some preparations that in the course of the experiment the perfusion pressure necessary in order to maintain a constant rate of perfusion must be raised gradually; this did not alter at all the amount of glucose absorbed.

It seems, therefore, justifiable to assume that the membrane characteristics remain unaltered with changes in perfusion pressure and perfusion rate within the limits of our experiments. Our discussion, however, must take into account the changes in capillary circulation associated with changes in circulatory rate. Schematically, there are different possibilities depending on whether or not the number of capillaries perfused with various pressures remains the same. If there is no change in the number of perfused capillaries and if, therefore, the area across which absorption takes place remains practically constant the rate of flow in each individual vessel will vary as the pressure, and a unit volume will stay in contact with the membranes across which the solute diffuses for a shorter length of time for higher perfusion rates, thus preventing the attainment of the same concentration as in the case of the "normal" perfusion rate.

If, on the other hand, an increase in pressure results in the opening of hitherto closed capillaries, the situation is different. There maybe a sufficient increase in the number of functional vessels to compensate for the increased rate of flow to the extent that there may be no change in the rate in each individual vessel, and it can accumulate the same concentration of glucose as previous to the pressure change. There is an increase in absorbing area, and no change in speed of flow in individual vessels. The rate of absorption will then be proportional to the rate of perfusion and the glucose concentration will be constant.

The opening of new vessels may not, of course, be sufficient to fully compensate for the increased pressure, in which case the rate of flow in individual vessels will be somewhat speeded up by increased pressure, but not to the same extent as when no change in the capillary bed occurred.

That is, there is an increase in absorbing area, but also an increase in rate of flow in individual vessels, so that while the total amount of glucose absorbed by the circulation increases, the *concentration accumulating* suffers a decrease.

There is still another condition which might occur. An increase in pressure might result in the opening of a relatively larger number of previously closed capillaries than needed to maintain the speed of flow in individual capillaries constant, so that there would be a slowing down of flow in individual vessels, in spite of an increased flow in the circulation as a whole. This, with the overlarge increase in absorbing area accompanying it, would result in a disproportionately large increase in the amount of glucose absorbed, with an actual increase in the concentration of glucose accumulating in the perfusion fluid.

The concentrations and total amounts of glucose found experimentally indicate that all the above discussed conditions occur in various preparations. The following summary gives the theoretical cases, and the number of experiments which agree with them:

1. Absorbing area constant. No change in the number of functional capillaries: increased pressure gives increased speed of flow in individual vessels, with decreased concentration of glucose and no change in total amount absorbed per unit time. Four experiments gave this result.

2. Absorbing area increases with increasing pressure, because of the opening up of new capillaries. Three possibilities present themselves:

a. Rate of flow in individual vessels constant: concentration of glucose constant, amount absorbed proportional to rate of flow. Seven experiments gave this result.

b. Rate of flow in individual vessels increased. Concentration of glucose declines somewhat, but amount absorbed is somewhat increased by increased rate of flow through the whole system. Twelve experiments gave this result.

c. Rate of flow in individual vessels decreased with increase in pressure and total flow. Raising pressure gives an increase in concentration and a disproportionate increase in amount absorbed. One experiment gave this result.

Decreases of pressure will, of course, give converse results in all groups discussed in 1 and 2.

The rule which governs all cases is that the concentration of glucose accumulating is inversely proportional to the speed of flow in the individual vessels of the absorbing system and directly proportional to the total absorbing area. According to our results, this speed of flow, due to variations in the reactions of the capillaries to changes in perfusion pressure may have almost any relation to the pressure applied.

Direct observation of the capillary bed to determine whether or not

changes in pressure produced these changes in number of functional capillaries and in the rate of flow in individual vessels proved to be impossible in our preparation. But the effect of pressure changes on the capillaries of the lungs has been observed by Wearn, Barr, and German (1926), who found that a rise in blood pressure produced by clamping the aorta resulted in the opening of new capillaries and even arterioles in a field under observation, and that the fall in pressure following the release of the aorta resulted in the closure of many capillaries and at times arterioles.

Still more pertinent are the observations of MacGregor (1933). He produced, by the injection of adrenalin, acetyl choline, and histamine, increases and decreases in the total pulmonary flow, while keeping the capillary circulation under direct observation. He found that no regular change in the speed of flow in individual capillaries of the lung resulted: an increase in total circulation led sometimes to an increase, sometimes to no change, and sometimes to a decrease in the rate of flow in individual capillaries. Decreases in total circulation led to similar results.

Our observations on the rate of glucose absorption under the influence of variations in the total perfusion rate led us to assume that the speed of flow in the individual capillary may be increased, decreased or unchanged when the total flow is increased. Furthermore, it was assumed that in order to account for the variability in the capillary flow the total absorbing area may or may not be increased by opening up of new capillaries under increased perfusion pressure. Both suppositions deduced from our absorption studies are verified in the studies of capillary flow in the lungs under similar conditions by Wearn and co-workers, and MacGregor. Therefore, it may be said that the influence on the absorption rate resulting from alterations in the total blood flow is determined by the state of the capillaries in the gut and their reaction to changes in perfusion pressure.

SUMMARY

Systematic studies on the influence of the total circulatory flow through the gut on the absorption rate of glucose are reported. The gut of Rana esculenta is perfused with isotonic glucose solution, the mesenteric artery with Ringer's and the glucose concentration and its amount in Ringer's leaving the portal vein is estimated. It is found that in some preparations wide variations in total flow brought about by changes in perfusion pressure resulted in corresponding changes in the amount of glucose absorbed. In these cases the *glucose concentration* in the blood vessels remained unchanged in spite of considerable changes in the amount of Ringer's which circulated through the gut per unit of time. In other cases the *amount of glucose* absorbed remained the same at various degrees of circulatory rate showing that the glucose concentration varied inversely with the rate of circulatory flow. The various types of absorption rate in regard to con-

centration and amount observed under conditions of increased or decreased circulatory rate are explained on the basis of the assumption that the state of the capillaries in the gut and their reaction to changes in perfusion pressure varies in different preparations. If increased pressure results in an opening up of new capillaries, the rate of flow in the individual capillaries being unaltered, the concentration remains the same and the total amount absorbed varies in proportion to the perfusion pressure. If, however, no change in the number of functioning capillaries occurs with change in perfusion pressure, the rate of flow in the individual capillaries is directly proportional to the perfusion pressure. Then the glucose concentration in the blood vessels is inversely proportional to the total rate of flow but the total amount absorbed remains constant.

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ASCITES AND OTHER EFFECTS OF LARGE SALINE INJECTIONS

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When a saline, isotonic with the blood of an animal, is injected intravenously, much of the fluid soon leaves the blood stream. It is not unreasonable to expect, when large amounts are injected, that some of the fluid may transude into the various serous cavities, especially the peritoneal cavity. A considerable number of papers have been published on the effects of such large injections of saline. Many of the investigators were concerned primarily with the possibility or impossibility of producing visible edema by hydremic plethora and with the comparison of this edema with that occurring in kidney disease. It is unfortunate that in the literature the presence of ascites and certain other facts concerning the distribution of fluid are for the most part only mentioned in a qualitative manner.

Cohnheim and Lichtheim (1877) described the effects of massive saline injections. These authors, in common with most of the early workers, used a 0.6 per cent sodium chloride solution instead of the 0.9 per cent solution of our time. Apparently in most cases saline was continuously injected by vein until finally death of the animal resulted, and it was often necessary to inject 60 to 70 per cent of the body weight in order to bring about death. The course of events is graphically described in Cohnheim's *Lectures on General Pathology* (1889). The conclusions which are important here may be summarized as follows:

1. Most secretions were enormously increased. Saliva flowed abundantly from the dogs' jaws. During the experiment repeated watery evacuations took place from the intestines; and on examination after death, the stomach and intestines were found distended with turbid fluid. Bile production is stated to have been increased.

2. There was ascites. Cohnheim states that the "peritoneal cavity never fails to contain a considerable portion of clear fluid." No more definite information however is given.

Since Cohnheim and Lichtheim published in 1877, a goodly number of papers referring to this subject have appeared (Gärtner, 1883; Dembowski, 1885; Francotte, 1888; Dastre and Loye, 1888, 1889; Johansson and Tigerstedt, 1889; Sanotzky, 1891; Pisenti, 1891; Raum, 1892; Sherrington and Copeman, 1893; Knoll, 1895; Bosc and Vedel, 1896; Magnus, 1899; Selig,

1911; Roger and Garnier, 1913; Tsurumaki, 1923; Senga, 1924–25). It is not possible here to discuss the details of these papers. In none of them, just as in Cohnheim's work, is there a quantitative study of the serous transudates in hydremic plethora.

In 3 papers some quantitative data on ascitic fluid in hydremic plethora are available. Albu (1901) in a few scattered protocols records the volume of fluid in the peritoneal cavity. Engels (1904) in experiments in which the quantity of saline involved, after allowing for urine secretion, was apparently about 13 per cent of the body weight, found very little fluid in the peritoneal cavity. It is of interest that the animals were deprived of water for 4 days preceding the experiment; hence they must have been severely dehydrated. Bogert, Underhill, and Mendel (1916) in some work concerned mainly with smaller quantities, made a few injections of Ringer's solution in amounts equal to 20 per cent of the body weight, and observed only small quantities of ascitic fluid.

Mention should be made of some work, which has appeared recently (Lyon, Shafton, and Ivy, 1929; Ivy, 1931; Barry, Shafton, and Ivy, 1933). It was found that the survival period of nephrectomized dogs could be prolonged by giving Ringer's solution subcutaneously, and that general anasarca, ascites, hydrothorax, and hydropericardium were thereby produced.

In the present research quantitative measurements have been made on the amounts of fluid occurring in the serous cavities as a result of intravenous injections of Ringer-Locke's solution in quantities up to $\frac{1}{4}$ of the body weight. Certain other questions, concerning the distribution of fluid under such conditions, have been investigated, such as, for example, the amounts of fluid appearing in the stomach and intestines and the rapidity with which the fluid leaves the blood stream.

EXPERIMENTAL. A. *Procedure.* Ringer-Locke's solution was injected into adult dogs in quantities up to 25 per cent of the animal's weight. The kidney vessels had been previously ligated to prevent water loss by way of the urine. Fluid present in the peritoneal cavity, retroperitoneal tissues, pleural cavity, pericardial cavity, stomach, small intestine, large intestine, and gall bladder was then measured. Since it was desirable to make these measurements under conditions of equilibrium, the animals were not killed or the measurements made until 4 or 5 hours after the injection. This period allowed equilibrium to occur between blood and tissue spaces. The amount of fluid remaining in the blood at various times was judged by means of hemoglobin determinations. The details of the procedure follow below.

One week previous to the experiment the animals were given carbon tetrachloride followed by magnesium sulphate, in order to free them of intestinal parasites. They were fasted for a period of 72 hours before the experiment, and no water was allowed during the last 10 hours.

The animals were anesthetized with sodium phenobarbital (about 125 mgm. per kgm. intravenously). The renal arteries and veins were ligated, and the spleen was removed in order to prevent alterations in hemoglobin due to this organ. After this surgery, the abdominal wound was carefully and completely closed by linen sutures. Arterial pressure was recorded from the carotid artery by one of two methods. In some of the experiments the usual type of cannula, with 4 per cent sodium citrate in 0.6 per cent sodium chloride solution as an anticoagulant, was used; while in others a cannula described by Lamson (1920), in which no anticoagulant is necessary, was employed.

Hemoglobin was relatively determined by the method described by Scott (1917).

The saline used was a Ringer-Locke's solution, whose composition is given in Bayliss' text-book (1924).

NaCl	0.9	$NaHCO_3$	0.03
KCl	0.042	Water	to 100
$CaCl_2$	0.024		

This solution, warmed to about 38°C., was introduced into the left external jugular vein at a rate of 1.67 cc. per kgm. of body weight per minute. This speed is greater than that advised by Dastre and Loye (1889), but no untoward results were noted.

During the entire experiment, the animal was kept warm. In most cases the dog's temperature was observed by means of a rectal thermometer, and was maintained at approximately 101°F. In Schäfer's text-book (1898), there are quoted from the literature normal average values for the rectal temperature of dogs varying between 100.2°F. and 101.8°F. The maintenance of a normal temperature is important (Calvin, Smith and Mendel, 1933).

After the saline injection, a period of 4 or 5 hours was allowed to elapse before the animal was killed. Then the animal was bled to death from the right femoral artery, after which the various fluid quantities mentioned above were measured. Cotton was used in making these measurements. A mass of it, contained in a beaker, was first weighed. The fluid in the cavity or viscus in question was removed by soaking it up with some of this weighed cotton, wiping the walls as dry as possible. Finally the cotton, this time containing the fluid, was again weighed in its beaker, thus allowing the weight of the fluid to be calculated. The estimation of the fluid in the retroperitoneal tissues occasions some difficulty and inaccuracy. The peritoneum was torn, and as much fluid as possible was expressed from the loose underlying tissue.

B. *Results.* 1. *Fluid in the serous cavities and in the digestive tract.* When quantities of Ringer-Locke's solution equal to 17.5 or 25 per cent of the body weight were introduced into the circulation, there was a considerable production of ascites; and, in contrast, only relatively small amounts of fluid were found in the other serous cavities or in the stomach and small intestine. The data are recorded in table 1, all the results being expressed as percentages of the body weight. A series of 6 control experiments (table 2), in which the procedure was the same as outlined above except that no saline was given, was also performed. In these control experiments a period of about 6 hours was allowed to elapse between the surgery (ligation of renal vessels, removal of spleen, etc.) and the final bleeding of the animal. Measurements of fluid quantities were made as before. When the percentages from these control animals are subtracted from the

TABLE 1

Fluid found in the serous cavities and in the digestive tract

(The results are expressed as percentage of the body weight)

EXP. NO.	PERITONEAL CAVITY	RETROPERITONEUM	STOMACH	SMALL INTESTINE	LARGE INTESTINE	GALL BLADDER	PLEURAL CAVITY	PERICARDIAL CAVITY
Injection of 17.5 per cent of the body weight of Ringer-Locke's solution								
	per cent	*per cent*		*per cent*		*per cent*	*per cent*	*per cent*
1	1.030	0.300	0.010	0.440	Feces	0.150	0.190	0.010
2	3.614	0.524	0.559	0.607	Feces	0.124	0.172	0.034
3	1.528	0.600	0.128	0.136	Feces	0.184	0.096	0.036
4	3.622	0.685	0.090	0.595	Slight diarrhea	0.171	0.081	0.005
5	2.073	0.409	0.045	0.264	Feces	0.150	0.173	0.000
Av...	2.373	0.504	0.166	0.408	—	0.156	0.142	0.017
Injection of 25 per cent of the body weight of Ringer-Locke's solution								
6	5.012	0.259	0.253	0.418	Feces	0.147	0.406	0.006
7	4.854	0.427	0.379	0.488	0.026	0.118	0.292	0.013
8	3.255	0.386	0.110	0.766	Feces	0.041	0.262	0.014
9	4.504	0.808	0.152	0.936	Feces	0.128	0.720	0.008
10	4.756	0.447	0.309	0.520	Feces	0.146	0.382	0.024
Av...	4.476	0.465	0.241	0.626	0.026	0.116	0.412	0.013

TABLE 2

Control animals. The figures are the averages of 6 experiments

	PER CENT OF BODY WEIGHT OF FLUID
	per cent
Peritoneal cavity	0.251
Retroperitoneum	0.139
Stomach	0.067
Small intestine	0.249
Large intestine	0.005
Gall bladder	0.113
Pleural cavity	0.063
Pericardial cavity	0.007

corresponding values for the injected animals, a more exact idea is obtained as to how much of the accumulation of fluid was due to the hydremic plethora itself. These differences are presented in table 3.

It is interesting to express these results as fractions—that is to calculate what fraction of the injected fluid appeared in the serous cavities and other

organs measured. This is done in table 4. Thus, in the case of injections of 25 per cent of the body weight of Ringer-Locke's solution, a quantity equal to about $\frac{1}{6}$ of the injection appeared in the peritoneal cavity; and only relatively small proportions occurred in the other serous cavities or in various sections of the gastro-intestinal tract—thus about $\frac{1}{70}$ appeared in the pleural cavity, and the same fraction was found in the small intestine and also in the retroperitoneal spaces. Of the total amount of saline injected, approximately $\frac{1}{5}$ was accounted for by the above measurements.

TABLE 3

Percentages (of body weight) of fluid found in the serous cavities and in various parts of the digestive tract in hydremic plethora, from which percentages obtained in control experiments, with no saline injection, have been subtracted

The average values have been used

	17.5 PER CENT INJECTED	25 PER CENT INJECTED
Peritoneal cavity	2.122	4.225
Retroperitoneum	0.365	0.326
Stomach	0.099	0.174
Small intestine	0.159	0.377
Gall bladder	0.043	0.003
Pleural cavity	0.079	0.349
Pericardial cavity	0.010	0.006

TABLE 4

Fraction of the quantity of fluid injected appearing in the serous cavities and in the various parts of the digestive tract in hydremic plethora

	17.5 PER CENT INJECTED	25 PER CENT INJECTED
Peritoneal cavity	0.121	0.169
Retroperitoneum	0.021	0.013
Stomach	0.006	0.007
Small intestine	0.009	0.015
Gall bladder	0.002	0.0001
Pleural cavity	0.005	0.014
Pericardial cavity	0.001	0.0002
Total	0.165	0.2183

In all of the above experiments an attempt was made to measure the fluid in the large intestine. Feces were very frequently present, rendering exact determination of fluid impossible. However, the feces were almost invariably solid and dry, with no indication of excess fluid. Diarrhea did not occur except in one or two experiments, which were rejected for this reason.

Saliva was not measured quantitatively. No profuse amount of secretion was seen dripping from the jaws.

It should be emphasized that in the preceding experiments the action of the

kidneys was removed by ligation of the renal arteries and veins. In some of the preliminary experiments, instead of ligating the renal vessels, the ureters were tied. This procedure resulted in the formation of an astonishingly large amount of fluid in the retroperitoneal tissues about the kidney and ureter, when hydremic plethora was induced. The appearance upon opening the abdominal cavity was striking; the tissues were ballooned out by the fluid, and, when cut, presented a jelly-like consistency. These facts are mentioned by Magnus (1899), who suggests that the fluid was forced through the walls by the peristaltic contractions of the ureters. However this may be, in hydremic plethora, the pressure in the ureter may rise markedly. In one experiment, in which the right ureter was ligated and the left ureter connected with a mercury manometer, on injection of 25 per cent of the body weight of saline at the usual rate, the ureteral pressure rose as high as 159 mm. Hg, the mean blood pressure at this time being 188 mm. Hg.

It is to be remembered, in comparing the above results with those of Cohnheim, that the amounts of fluid injected by the author were smaller than those used by Cohnheim.

Roger and Garnier (1913), using nephrectomized rabbits, made measurements on the amount of material found in the small intestines in injections of Locke's fluid up to 52.1 per cent of the body weight. Their results agree fairly well with those obtained above, provided that one takes into consideration the fluid which might normally, without any saline injection, be expected to be present in the small intestines and also the fact that most of the injections of Roger and Garnier were larger than those used in the present research. No mention is made of a period of fasting previous to the experiment. These authors found no increased ascitic fluid in this series of nephrectomized animals except in one case.

MacCallum (1904), in experiments on nephrectomized rabbits, found larger amounts of fluid secreted into the stomach and small intestine in hydremic plethora than the author. Experimental conditions may account for this difference (e.g., in MacCallum's experiments the intestine and stomach were cannulated, m/6 or m/8 sodium chloride solution was used instead of the Ringer-Locke's solution employed by the author, morphinized rabbits were used instead of dogs anesthetized with luminal, etc.).

2. *Hemoglobin concentration.* Hemoglobin concentration was closely followed with the idea of determining how rapidly the fluid leaves the blood stream. Perhaps certain inaccuracies are involved in thus estimating the degree of dilution of the blood by means of hemoglobin determinations. It is to be remembered, however, that all the animals were splenectomized thus obviating any error from this source.

It was first necessary to determine the variation of hemoglobin in control experiments. In table 5 are the hemoglobin values at the end of control experiments, in which the procedure was carried out exactly as outlined above except that no saline was injected. The hemoglobin here, as in all cases, is expressed as per cent of the first sample. Thus, over a period of 6 hours, there was a concentration of about 5 per cent. This change should be considered in the interpretation of hemoglobin changes in the experiments on hydremic plethora.

The data from the experiments in which Ringer-Locke's solution was

injected may be seen in table 6. Immediately after the completion of the injection, the hemoglobin concentration rose sharply. Later on the fluid left the blood stream less rapidly, and so after 3 or 4 hours the rate of change of hemoglobin concentration was rather small. The average values for the 25 per cent injections are plotted in figure 1.

Even after a period of 5 hours, however, using the hemoglobin as an indicator, it was found that some of the saline still remained in the blood

TABLE 5

Variation of hemoglobin concentration in luminal anesthesia

Hemoglobin is expressed as per cent of the first sample

LENGTH OF PERIOD COVERED BY HEMOGLOBIN READINGS	HEMOGLOBIN PER CENT AT END OF PERIOD
hours	*per cent*
5½	100
7	102
6	105
6	106
6	110
6	106

TABLE 6

Concentration (relative to pre-injection value) of hemoglobin at various periods after the completion of injection of Ringer-Locke's solution

These figures are the averages of all the injection experiments

	17.5 PER CENT INJECTION	25 PER CENT INJECTION
	per cent	*per cent*
Hb per cent at end of injection	67.5	68.3
15 minutes later	74.0	73.4
½ hour	78.7	77.9
1 hour	80.9	81.3
2 hours	84.0	85.5
3 hours	86.4	88.7
4 hours	87.1	90.3
5 hours		91.6

vessels. Thus 5 hours after the end of the injection of a quantity of fluid equal to ¼ of the body weight, the hemoglobin percentage was about 92 per cent (average of 5 experiments). It must also be remembered that control experiments indicated a tendency toward concentration.

These findings are in accordance with those of Calvin, Smith, and Mendel (1933) who, using much smaller quantities of 0.95 per cent sodium chloride solution (amounts equal to the blood volume—i.e., 8.14 per cent of the

body weight), found that some of the fluid remained in the blood even after 4 hours. Sanotzky (1891) studied the changes in the water content of the blood after injections of 0.6 per cent sodium chloride solution in quantities

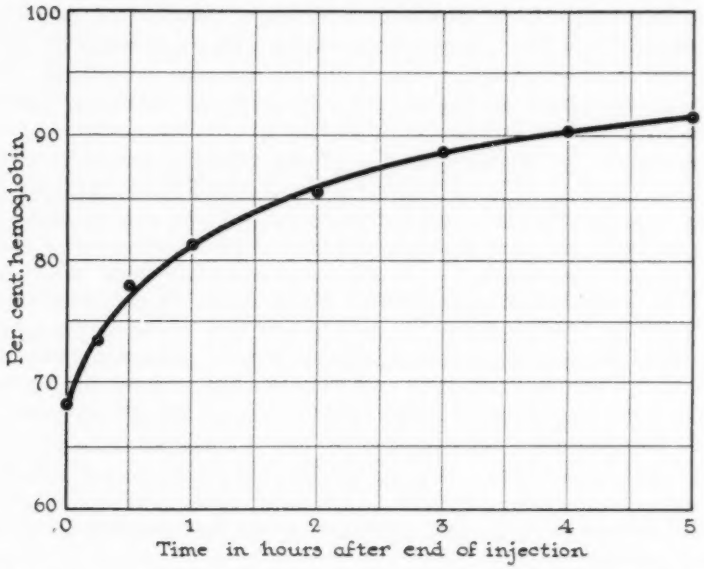

Fig. 1. Variation in hemoglobin concentration following the injection of a quantity of Ringer-Locke's solution equal to $\frac{1}{4}$ of the body weight. A composite of 5 experiments.

TABLE 7

Arterial blood pressure at the beginning and at the end of the experiments

Averages for the various series are given

	ARTERIAL PRESSURE	
	At beginning	At end
	mm. Hg	*mm.* Hg
Control experiments with luminal anesthesia..	122	116
Injection of 17.5 per cent of the body weight...	107	104
Injection of 25 per cent of the body weight	112	116

up to 300 per cent of the blood volume. His results are somewhat difficult to interpret because of the rather large proportion of blood removed from the animals in sampling.

3. *Arterial and venous blood pressure.* Arterial pressure was recorded

in all cases. This pressure serves to some degree as a check on the condition of the animal. Experiments in which the pressure was lowered to a marked extent were rejected. The pressure at the end of the experiment was in some cases somewhat higher and in some cases somewhat lower than at the beginning. If the pressures are averaged, however, the values are approximately the same, as the accompanying table shows (table 7).

In some early experiments, venous pressure was also determined. The pressure rose markedly during the injection; and it was still elevated even 4 or 5 hours after the completion of the injection.

Discussion. In the consideration of the preceding results, it is important to keep in mind the conditions under which these data were obtained. In the first place an anesthetic was used, and it is possible that the anesthetic used, luminal, may influence water distribution. Chloroform and ether anesthesia, for example, may have some effect—see Dastre and Loye (1889) and Magnus (1899). Furthermore, the peritoneal cavity was opened in order to ligate the renal vessels and to remove the spleen; and, since these were acute experiments, aseptic precautions were not observed. These circumstances may be suspected to have some effect, especially on the quantity of ascitic fluid produced. Finally, an anuria of some 7 hours duration was present at the end of the experiments, since the renal vessels were ligated. It is conceivable that this factor may influence water distribution (Magnus, 1899). All these conditions are mentioned as possible factors exerting some influence on the fluid distribution in the present experiments.

SUMMARY

When 17.5 or 25 per cent of the body weight of Ringer-Locke's solution was injected into dogs at a rate of 1.67 cc. per kgm. per minute, there was a marked increase in the fluid found in the peritoneal cavity and relatively smaller increases in the fluid in the other serous cavities, in the stomach, intestines, gall bladder, and retroperitoneal tissues. The averaged results (expressed as per cent of the animal's weight and corrected by controls) are as follows:

	17.5 per cent injected	*25 per cent injected*
Peritoneal cavity	2.122	4.225
Retroperitoneum	0.365	0.326
Stomach	0.099	0.174
Small intestine	0.159	0.377
Gall bladder	0.043	0.003
Pleural cavity	0.079	0.349
Pericardial cavity	0.010	0.006

Some fluid remained in the blood even 4 or 5 hours after the end of the injection, as shown by hemoglobin determinations.

These results were obtained on animals who were anesthetized with luminal, whose renal vessels were ligated, and who were splenectomized.

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REACTIVE HYPEREMIA: RELATION OF DURATION OF INCREASED BLOOD FLOW TO LENGTH OF CIRCULATORY ARREST

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It has long been known that upon the release of a complete obstruction of the circulation of an extremity there supervenes a deep flushing of the skin of the limb distal to the point of circulatory arrest. Experiments performed by various workers with the plethysmograph have shown that the flushing is accompanied by an increase in limb volume. These manifestations have been found to be transient and are believed to be due to a general vasodilatation of the vessels of the skin, the muscles and other structures within the involved area. This phenomenon is known as reactive hyperemia.

Although a considerable amount of work has been done upon the problem, there still exists in the literature of the subject a difference of opinion concerning the relation of the duration and intensity of the reaction to the length of the circulatory arrest. The opposing points of view are expressed by Lewis and Grant (1) on the one hand and Goldblatt (2) on the other. Since these workers have done most in recent times to advance our knowledge of this phase of reactive hyperemia our review of the literature will be limited to their work.

Lewis and Grant made their careful observations upon the forearm and leg of the unanesthetized human being. Changes in the color of the skin, in the limb volume as recorded by a specially prepared plethysmograph, and in the volume flow of blood were noted. The latter was determined by the method of Hewlett and Zwaluwenburg (3), which consists of obstructing the venous return proximal to the involved area by applying through a 12 cm. armlet, suddenly and for a few seconds only, a pressure of 55 to 70 mm. Hg and measuring the rate of increase in limb volume as recorded by the plethysmograph. These authors found that the data which were ob-

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tained by the visual, the limb volume, and the blood inflow methods were in agreement. Among other things, they observed that the duration and intensity of the hyperemia were approximately proportional to the length of circulatory arrest. The duration of the reaction was found to be one-half to three-fourths as long as the period of occlusion. Only rarely was the duration of the former equal to that of the latter. It was found also that the end point of the reaction could not be determined with absolute certainty by any of the three methods.

On the other hand, Goldblatt, working with the denervated limbs of anesthetized animals, observed that the phenomenon increased in duration and intensity for periods of circulatory arrest up to and including 2 to 3 minutes. Following the longer periods of occlusion the tendency was for the reaction to decrease. In several instances "occlusions lasting for fifteen minutes and longer were not followed on release by any appreciable return of limb volume beyond normal." The reaction was measured chiefly by changes in limb volume as recorded by a water-containing glass plethysmograph connected by an air line to a sensitive piston recorder.

Since the full appreciation of the alterations which occur in the volume flow of blood is necessary for the complete understanding of the phenomenon of reactive hyperemia, and since the observations of Lewis and Grant and of Goldblatt are not in agreement, it seemed to us that a more perfect picture of the process might be gained by making direct measurements of the changes which occur in the volume flow of blood. This we have undertaken to do. The present paper deals with the study of the relation of the duration and intensity of the increase in blood flow to the length of the period of circulatory arrest, in the anesthetized and the unanesthetized animal with the nervous system of the extremity intact.

METHOD. Rather large, apparently healthy dogs, weighing between 15 and 21 kgm. were used. Heparin was given as an anticoagulant (30 mgm. per kgm.). The blood flow was measured at first through the iliac artery. Later the superficial femoral arterial flow was taken as representative of the general flow to the extremity and the circulation was arrested by means of a tourniquet placed about the limb just distal to the insertion of the stromuhr cannulae and just tight enough to occlude the arterial supply to the extremity (fig. 2). The blood flow was measured by a modified Ludwig-Stolnikow stromuhr. Studies were made upon sixteen anesthetized and three unanesthetized animals. Observations were carried out on a single animal for periods varying between one and three and one-half hours. No period of tourniquet occlusion of the circulation lasted longer than ten minutes.

In the anesthetized animals the systemic blood pressure was taken from the carotid artery, while in the unanesthetized animals it was obtained through a side arm connection with the stromuhr inlet cannula. Sodium

barbital (250 to 260 mgm. per kgm. intraperitoneally) and sodium amytal (35 to 45 mgm. per kgm. intravenously in divided doses beginning with 35 mgm. per mgm.) were used as anesthetics in separate experiments. Occasionally they were supplemented by small amounts of ether. The unanesthetized animals were trained for several weeks to lie quietly. At the time of the experiment they were given $\frac{1}{4}$ to $\frac{1}{2}$ grain of morphine sulphate subcutaneously and the femoral artery was exposed aseptically, using local (1 per cent) novocaine infiltration. All three recovered from the effect of the procedure.

The blood flow apparatus which was used incorporates the oil piston, double chamber stromuhr of Ludwig (4), with the Y inlet-outlet of Stolnikow (5), and the electromagnetic valve control of Pavlov (6). From Gesell (7) we obtained the idea of the blood electrolyte activation of a motor which led to the development of our automatic control. A preliminary demonstration of the stromuhr was made by one of us (M. L. M.) before the Thirteenth International Physiological Congress (8). Its principles later were incorporated in a pump system which was devised by Hemingway (9) and recently have been modified by Still, Bennett and Scott (10). Our own description of the mechanism has been delayed by unsatisfactory experiences with electrolytes. Blood coagulates upon the electrodes when it is used as an activator and the copper sulphate-oil interface as used in the demonstration fails to give a sharp contact at all times, either with copper or platinum electrodes. Since the appearance of the initial description by Bennett and Still (11) we have adopted a modification of their polarized relay control. For the purpose of measuring the arterial blood flow in units of 3 to 6 cc. we have not found their oil-copper sulphate-chloroform method superior to our own as far as resistance and accuracy are concerned, providing the number of reversals does not fall below 10 to 15 per minute. When the reversals are rapid our experience has favored our mercury method.

The manometer unit and valve control are shown in figure 1. The former is composed of a rounded M-shaped glass tube with the outer arms acting as vertical receiving chambers and the central U forming the governing electrode chamber. The latter is placed in a plane in front of and parallel with the plane of the receiving chambers. A little above the middle of the arms of the U, and at the same horizontal level, each arm is expanded to form a cylindrical enlargement with vertical sides 2 to 2.5 cm. long. The diameter of this cylinder is varied with the delivery desired. Into the upper neck of each expansion there is sealed a tungsten wire to the inner tip of which has been welded a semi-flexible platinum electrode (no. 21 gauge, Brown and Sharp). The latter extends obliquely downward to the vertical axis of the U tube arm and then vertically downward in this axis to end a little above the line of bisection of the vertical sides of the

enlargement. The tip of the electrode is thus located in the center of the expanded cylinder.

At the top of each arm of the U tube and centered over its vertical axis a 6 mm. glass tube is sealed. The seal is expanded over the bend to eliminate

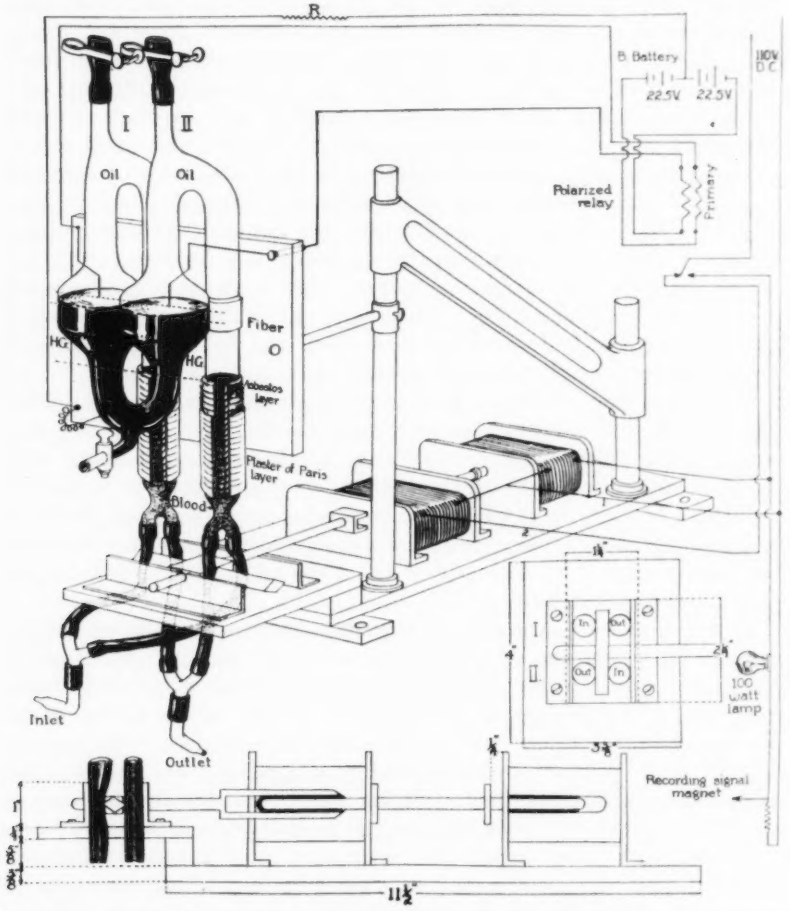

Fig. 1. Diagram of stromuhr, control system and heating elements. Toy transformer is not shown.

air pockets. Over the tips of these tubes semi-pressure rubber tubing are placed and sealed by Day pinchcocks. Through the tubes, so placed, a fine (21 gauge) platinum wire hook or heel can be passed to elevate or depress the platinum electrodes. It is essential that the electrode tips be

placed in the same horizontal plane. A stopcock is placed in the bottom of the U to allow for inflow and outflow of mercury. Near it a straight tungsten-platinum electrode is sealed into the tube. On the bottom of each receiving chamber an inverted Y tube is sealed with the arms in a vertical plane perpendicular to the plane of the receiving chambers. Rubber tubes are connected with the arms of these Y tubes and passed through the valve as shown. Below the valve the rubber tubes in-I and in-II are joined by a glass Y tube which in turn is joined to the inlet cannula. Tubes out-I and out-II are joined similarly and are connected with the outlet cannula. Rubber tubes pairs (in-I, out-II) and (in-II, out-I) are alternately compressed and released by the valve, which consists of two adjustable angle-iron stops and a horizontal, blunt, double knife edge (fig. 1). The latter moves in a straight line, and exerts pressure equally upon the tubes. It differs from the valve described by Barcroft (12) in which the knife edge moves in an arc. The knife edge rod is joined to the two solenoid armatures "in tandem," and by alternate activation of the solenoids the knife edge is moved back and forth. The length of stroke is altered by changing the distance between the solenoids.

For volume flow studies using a delivery of 3 to 6 cc. per filling the following materials are used: semi-pressure rubber tubing with a wall of $\frac{1}{8}$ inch and a lumen $\frac{3}{16}$ inch in diameter;[3] glass Y tubes, 6 mm. diameter, with limbs close together; receiving chambers, 16 to 18 mm. diameter; expanded cylinder 4 to 5 cm. diameter; 2 mm. stopcocks; no. 21 B. and S. platinum and medium sized tungsten wire for the electrodes; manometer solenoids, commercial type D.C., with pull of 3 to 4 pounds, of which one is the so-called "push-pull" type.

Controller system. The controller system consists of one Western Electric no. 226A polarized relay which takes its current from a B battery as shown and which works efficiently drawing a current of 12 to 15 milliamperes. The resistance, R, is adjustable so that as the voltage of the battery declines with use the amperage may be maintained. The stationary tips of the primary switch are rounded and will carry a current of 100 milliamperes without burning. Placing a condensor across each solenoid circuit will reduce the spark between the points of this switch.

Heating unit. The heating unit consists of a "toy" transformer and two cylindrical elements which may be slipped on and off of the receiving chambers. Each element is composed of an inner layer of asbestos and an outer layer of crinoline-plaster of paris, between which are a series of coils of resistance wire. A small portion of the circumference of each element is left incomplete so that the glass chambers may be visible and also so that the element may be slipped over the inlet-outlet arms, which have a spread wider than the diameter of the chamber. The toy transformer is of the

[3] All measurements refer to the inside diameter.

type which reduces a 110 volt A.C. current to a range of 1 to 32 volts. For a chamber tube 18 mm. in diameter we use 22 turns (210 cm.) of 24 gauge chromel A wire, 5 mm. apart. The voltage should be adjusted so that the element will maintain the temperature in the chamber between 38° and 40°C.

Preparation of the manometer unit. Clean mercury is run into the U tube through the lower stopcock until both electrode tips just touch its surface. The tips may be leveled by slight lateral movements of the fiber holder (fig. 1). Then the remainder of the unit is filled through the inlet and outlet tubes with a high grade, inert mineral oil, during which time the upper pinchcock controlled tubes are left open. If the oil forms a film on the mercury it cannot be used. Distilled water is now run about half way up into the receiving chambers and oil is forced out of the upper tubes into a temporarily connected receptacle. *The oil-water surfaces must be in the same horizontal plane, otherwise the instrument will not run smoothly.* Mercury is now withdrawn from the U tube in an amount equal to the desired delivery, and oil to replace it enters through the upper tubes. All pinchcocks and the stopcock are then closed, the temporary receptacle is removed, and the instrument is calibrated. Finally the distilled water is replaced by allowing a 0.9 per cent NaCl solution to run through the instrument for several fillings, and the inlet and outlet arms are then placed in their proper positions in the artery. (The manometer is cleaned as follows: concentrated alkali, wash with water, cleaning solution, wash with water, dry.)

Calibration. The mercury piston thrust which is optimum, ranges between 1.5 and 3.0 mm.[4] Before each experiment the instrument must be freshly filled and calibrated. The delivery in grams (or cubic centimeters) of distilled water for a given number of fillings (50 or 100) is determined at different speeds.[5] From these data the cubic centimeters per filling, as ordinates, are plotted against the fillings per minute, as abscissae, and the ascending straight line curve is drawn. Upon completion of the experiment the rate of fillings per minute throughout the desired parts of the experiment is determined and the amount delivered per filling is read directly from the curve, or is calculated by means of the following equations:

1. $m = \frac{y_2 - y_1}{x_2 - x_1}$ when m = the slope of the calibration curve, or the variation factor; y = the delivery per filling; x = the number of fillings

[4] With a mercury piston face of 5.0 cm. diameter the theoretical delivery is 1.9 cc. per 1.0 mm. of thrust. In reality the delivery varies directly with the number of fillings per minute. The function of this variation is a straight line curve.

[5] The speed of delivery is varied by changes in the outlet resistance and in the height of the pressure bottle. Time is measured by a stop watch.

per minute; and x_1y_1 and x_2y_2 are two different points on the curve. Now if the points are taken so that $x_1 = 0$, we have $m = \frac{y_2 - y_1}{x_2}$. The values for y_1, x_2, and y_2 are read directly from the curve.

2. When m and y_1 are determined it is then a simple matter to calculate the delivery per filling for any speed from the following equation: $y_2 = mx_2 + y_1$, when x_2 = the speed and y_2 = the delivery for that speed. For example, if $x_1 = 0$, $y_1 = 3.485$, $x_2 = 35$, $y_2 = 3.810$ as read from a calibration curve, then $m = \frac{y_2 - y_1}{x_2} = \frac{3.810 - 3.485}{35} = 0.00928$. Now if the delivery per minute is desired when the rate is 50 fillings per minute, we have from equation 2: $y_2 = 0.00928 \cdot 50 + 3.485 = 3.949$ cc. per filling; and the delivery per minute is $50 \cdot 3.949 = 197.5$ cc.

Efficiency. The error in recorded measurement averages well under $\pm$ 1.0 per cent with speeds up to 90 to 100 fillings per minute. At higher rates of speed it is increased somewhat. When high rates of flow are expected, large diameters, or longer mercury piston thrusts should be used rather than very rapid speeds of filling. The error in absolute delivery due to the resistance of the instrument has been rather difficult to determine but it seems not to be appreciable when reasonable speeds of filling per minute are used.

RESULTS. *Reactive hyperemia in the anesthetized animal.* At the inception of this study the animals were anesthetized with sodium barbital, intraperitoneally, the blood flow through the iliac artery was measured, and the occlusion of the circulation was accomplished by clamping the stromuhr inlet arm. Several of these preparations showed marked depression of the circulation. At first this was believed to be due to the opening of the abdominal cavity. We resorted, then, to the study of the blood flow through the superficial femoral artery, as representative of the blood flow through the limb and in order to prevent the possibility of a large collateral system supplying an adequate blood flow if the femoral artery alone were obstructed, a tourniquet was used to occlude the entire circulation of the part distal to the insertion of the cannulae (fig. 2). The tendency for the circulation to be depressed continued. It was largely relieved by substituting sodium amytal, intravenously, for the sodium barbital, although two of the eight preparations in which the sodium amytal was used were not very satisfactory.

Before switching to sodium amytal it was observed in a number of the early experiments that following the occlusion of the arterial circulation incident to the insertion of the stromuhr cannulae, the initial blood flow began at a very high rate and gradually fell to a much lower level where it became stabilized after a period of flow lasting nearly as long or longer than the period of occlusion, whereas later in the experiment nearly comparable

obstructions were not followed by a similar pattern. At first, we distrusted the effect of the insertion and use of the stromuhr, feeling that possibly some resistance factor which was not evident was shunting the blood through the collateral channels. Increasing experience demonstrated, however, that fundamental alterations in circulation were responsible, since, in certain of the experiments in which the circulation was

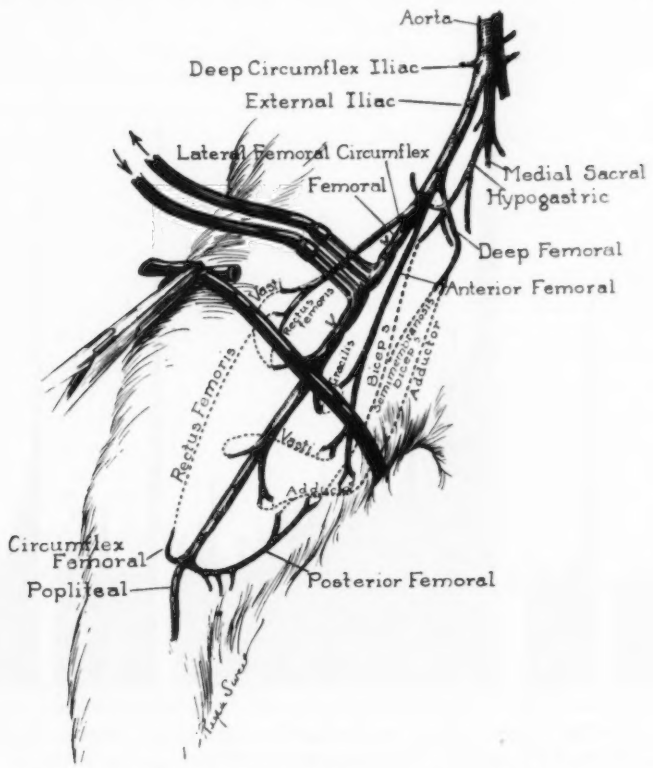

Fig. 2. Diagram showing position of the tourniquet and the insertion of the stromuhr to the circulation of the limb.

obviously seriously disturbed, the initial increase in blood flow following insertion of the cannulae also was absent. In addition, it was determined that repeated occlusions, especially of the longer durations, produced marked depressions of the circulation, with the result that the data obtained under such conditions were untrustworthy.

However, by using sodium amytal intravenously and only a few ob-

structions of the circulation per experiment several satisfactory experiments were obtained. In four of these the effect of obstructions lasting 10 minutes was compared with obstructions of 1 to 5 minutes. In two others, the obstruction time ranged between 15 seconds and 3 minutes. In all of these experiments the blood flow following the release of the obstruction was increased above the preceding basal flow and the increase tended to show an intensity and duration roughly proportional to the length of the period of obstruction.

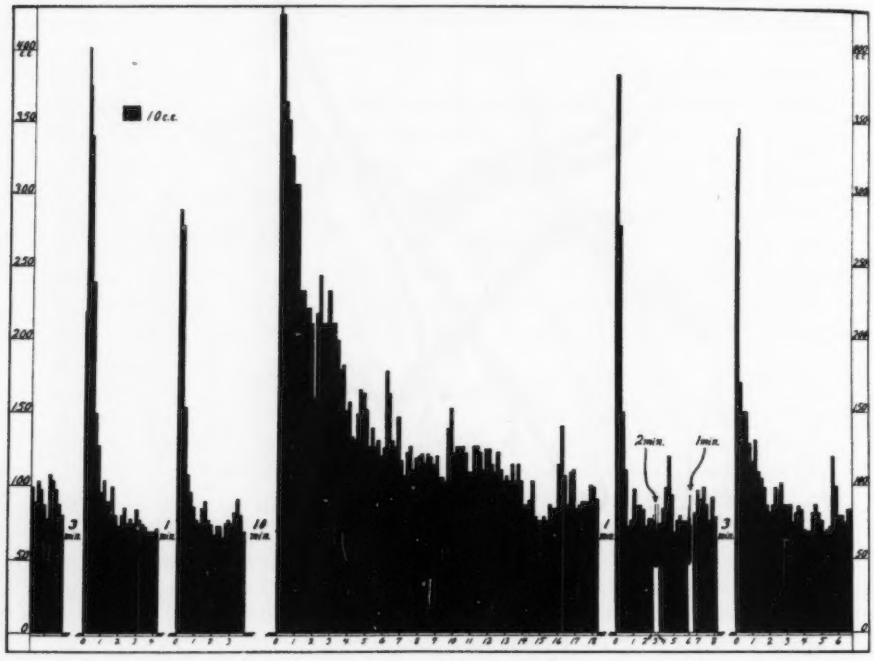

Fig. 3. Volume flow changes in the lightly anesthetized animal. Ordinates, cubic centimeters per minute. Abscissae, time in minutes.

The results obtained in the best of these experiments are presented in figure 3. The animal was a young, healthy, 9 months' old police dog, weighing 19.6 kgm., which had spent its entire life in the laboratory. A maximum of 45 mgm. per kgm. of sodium amytal, intravenously was used for anesthesia. The wink reflex was present. The general level of the carotid blood pressure varied between 186 and 190 mm. Hg. Respiration was quite deep and was associated with rather sharp fluctuations of both the carotid blood pressure and the femoral arterial blood flow. From a

study of the graph it is clearly evident that the intensity and duration of the hyperemia, as represented by the blood flow, are increased with the lengthening of the occlusion for periods of ten minutes or less. It is also evident that the duration of the phenomenon approaches 75 per cent to 100 per cent of that of the obstruction. The blood flow following the ten minute occlusion reached a low point 8½ minutes after onset, then rose moderately to a peak in the 10th and 11th minutes after which it fell to a lower stabilized level, comparable with the pre-occlusion flow, which was reached during the fifteenth minute of flow. This pattern differs somewhat from that seen in the unanesthetized animal.

Reactive hyperemia in the unanesthetized animal. In two of the three experiments performed in this series the data conformed rather closely with that obtained from the anesthetized animals. In the third, the duration of the increased blood flow following the ten minute occlusion was somewhat less, lasting six minutes with the peak of the brief rise occurring in the seventh minute and the return to normal developing in the eighth minute. This variation strengthened our idea concerning the relation of the state of the circulation to the pattern of the hyperemia for it was evident during the dissection of the femoral artery that the circulation of this animal was not normal, since frequent skipped pulse beats were very noticeable.

The results obtained in the best of the three experiments are presented in figure 4. The animal was well trained, in good health, and weighed 20.7 kgm. The record shows that the intensity and duration of the hyperemia increased with the length of the period of circulatory arrest. In contrast with the observations on the anesthetized animal it was found following the ten minute arrest that although the sharp fall in blood flow ended after about 7½ minutes of flow, the low point in the cycle occurred after 10½ minutes of flow. Moreover, the secondary, moderate rise was more definite and smoother and was somewhat later in leveling (about the 18th minute). The reason for this difference is not clear unless it is assumed that the vasomotor nerves do play a part in the process and that in the lightly anesthetized animal they were partly inhibited.

Discussion. The experiments reported in this paper demonstrate that in both the anesthetized and unanesthetized animal in which the circulation is not seriously disturbed, the intensity and duration of the phenomenon of reactive hyperemia is approximately proportional to the length of the circulatory arrest for periods of ten minutes or less. Although longer periods of obstruction have not yet been tried it seems probable that they too would produce a similar result, providing they did not exceed the time necessary to cause serious damage to the involved circulation. What this end point is has not yet been determined but it is more than ten minutes, and therefore is much longer than the 2½ to 3 minute period proposed by Goldblatt. It is our belief that the inability of this worker to demonstrate

the phenomenon following the longer periods of circulatory arrest was due to a disturbance of the circulation in his preparations sufficient to impair the reaction in a degree similar to that observed in some of our animals. This disturbance may have been the result of the anesthetic used, the nature of the preparation, or the use of too many obstructions in a single experiment.

Our findings confirm, therefore, the observations of Lewis and Grant upon the unanesthetized human being. Although we have not yet attempted to investigate the significance of Lewis' H substance, our work

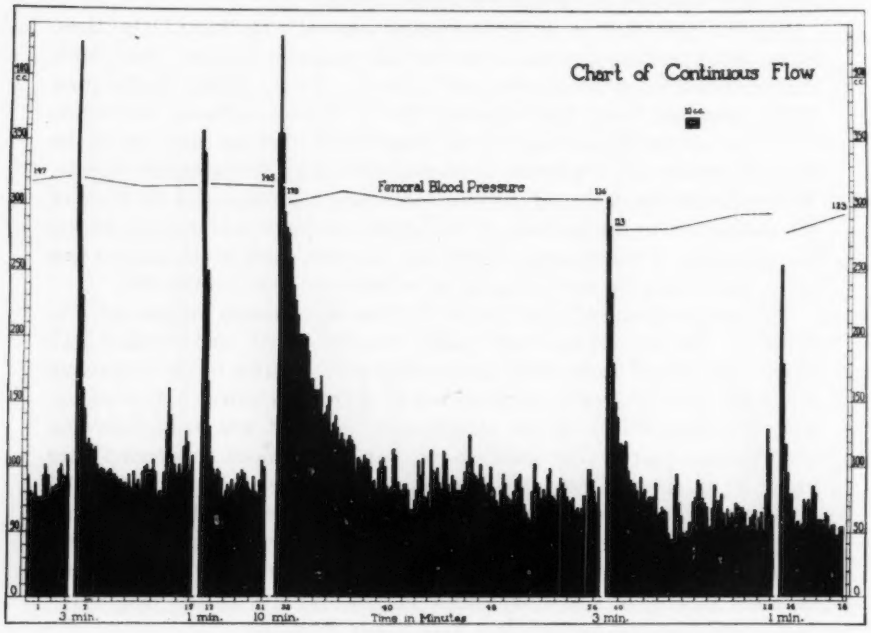

Fig. 4. Volume flow changes in the unanesthetized animal

shows that under proper conditions following the obstruction of the undamaged circulation to an extremity some factor is developed within the limb in such a manner that the duration and intensity of its influence increases with the lengthening of the period of the obstruction short of that which causes serious impairment of the circulation.

Our work has demonstrated also that the influence of the hyperemic factor lasts somewhat longer than can be demonstrated by the limb volume method. We believe further that the difference between the initial level reached in the anesthetized and unanesthetized animal may be related to

the paralysis of the vasoconstrictor nerves in the former. This idea is to be tested on the denervated limb of unanesthetized animals.

CONCLUSIONS

1. In the lightly anesthetized, and the lightly morphinized, unanesthetized dog with intact nervous system the release of a complete tourniquet obstruction of the circulation of an extremity is followed by a transient increase above the normal in the volume flow of blood through the extremity.

2. This phenomenon increases in duration and intensity with the lengthening of the circulatory arrest for periods ranging between 15 seconds and 10 minutes. Longer periods of arrest have not been studied.

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THE INFLUENCE OF THE THYROID ON THE ACTION OF GONAD-STIMULATING HORMONES[1]

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In a number of previous communications (Fluhmann, 1932a, c) certain important differences were demonstrated in the effects induced in immature rats by gonad-stimulating hormones obtained from anterior lobe material and from blood of pregnant women. It was also suggested that some of these variations were possibly due to the intervention of extraneous factors and not solely to differences in the action of the hormones on the ovaries of the test animals. Of these factors, the thyroid gland was considered of primary importance and an attempt has been made to determine to what extent it may influence the ovarian changes induced by gonad-stimulating hormones. For this purpose the effect of such hormones has been studied in immature rats which were either fed desiccated thyroid substance or in which a thyroidectomy had been performed.

The literature on this problem is somewhat limited, although many observations have previously been made on the effect of feeding desiccated thyroid substance or of thyroidectomy on the course of gestation or the estrual cycle of various laboratory animals. However, the work of Loeb and Friedman (1931) with the injection of gonad-stimulating hormones in guinea pigs suggests a relationship between thyroid and ovarian responses; Reiss and Pereny (1928) and Van Horn (1933) found a definite inhibition of the action of estrin in rat castrates following the administration of thyroid substance, while Van Horn (1933) could not demonstrate that thyroid feeding interfered with the action of a sheep anterior lobe extract on the ovaries of adult rats. It is also significant that the greatest ovarian response secured by Smith and Engle (Smith, 1932) from pituitary im-

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plants was in a thyroidectomized rat, and Schockaert (1931) has shown that the enlargement of the secondary sex organs in male rats given anterior pituitary injections is significantly increased by thyroidectomy. On the other hand, Loeser (1932) found in a small number of rabbits that thyroidectomy apparently did not interfere with the ovarian response induced by dried anterior lobe powder.

Extracts. The method employed in preparing the gonad-stimulating extracts was the same in all instances and consisted of an extraction with a sodium acetate-acetic acid buffer, pH 4.2 to 4.6, and reprecipitation of the active material with ethyl alcohol. The details have been given in previous reports (Fluhmann, 1932a, 1933a,c), as well as a full description

TABLE 1

Effect of various anterior pituitary gland extracts on the oxygen consumption of immature rats

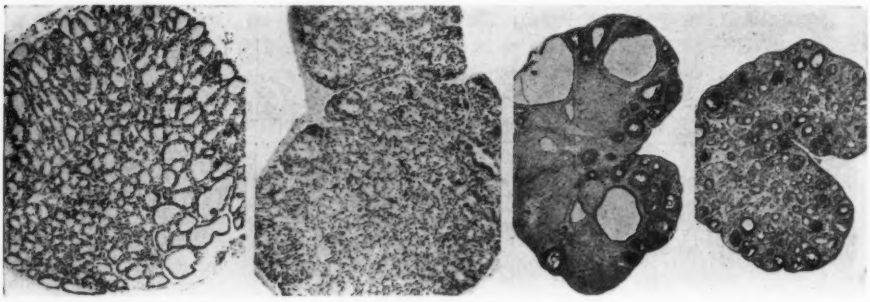
EXTRACT	DURATION OF EXPT. IN DAYS	NO. OF RATS	AVE. BODY WEIGHT	PER CENT INCREASE WEIGHT OVARIES	AVE. O_2 CONSUMPT. RATE	PER CENT OF NORMAL RATE
Controls		12			0.61	100
Human	5	5	45	237	0.57	94
Human	5	4	27	854	0.71	116
Human	15	9	54	146	0.68	111
Rat	5	7	35	620	0.79	130
Rat	10	7	43	488	0.68	111
Sheep	5	5	38	10	0.61	100
Sheep	20	3	50	10	0.83	136
Sheep	5	4	39	280	0.56	92
Sheep	5	3	36	120	0.76	125
Sheep	10	3	43	62	0.68	111
Sheep	10	10	62	312	0.88	144
Sheep	15	3	66	300	0.69	113

of the methods of administering the extracts, the procedure for determining per cent increases in organ weights, the rats employed, and the ovarian changes induced in immature rats in experiments of long and short duration.

The effect of the different extracts on the thyroid gland was studied by determining the oxygen consumption rate of the rats, as well as by histological examination. The oxygen consumption of the animals was estimated on the day they were sacrificed. Each animal was anesthetized with chloretone, a dose equivalent to 200 mgm. per kilo body weight being given intraperitoneally. A delay of at least one hour, when the rats were kept at a temperature of from 26° to 28°C., intervened before the rate was estimated. The amount of oxygen consumption was determined by placing the animal in a closed copper chamber surrounded by a water-jacket, and

kept at a constant temperature of from 26° to 28°C. This chamber contained soda lime to absorb carbon dioxide and water and the oxygen consumption was measured by means of a sensitive volume recorder. The rate was calculated in terms of cubic centimeters of oxygen per 100 square centimeters of body surface per minute. The average normal figure was obtained from the examination of 12 rats representing the age and weight range of the experimental animals.

The effect of various gonad-stimulating extracts made from human, rat and sheep anterior hypophyses on the rate of oxygen consumption of rats



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Figs. 1–4

Fig. 1. Photomicrograph of the thyroid gland of an immature rat given a moderate dose of an acid extract of sheep anterior pituitary glands. In spite of an increase in the rate of oxygen consumption the thyroid gland does not show any striking histological variation from the normal.

Fig. 2. Photomicrograph showing hyperplasia of the thyroid gland of an immature rat resulting from the administration of large doses of the sheep anterior lobe extract used in these studies.

Fig. 3. Ovary of a normal immature rat given an extract made from blood of pregnant women.

Fig. 4. Photomicrograph of an ovary of a thyroid-fed immature rat given an extract made from blood of pregnant women, and showing extensive production of interstitial tissue.

is given in table 1. A total of 63 rats was used and the results are expressed as the average obtained in small groups injected with various preparations of different strength. Since these studies were concerned primarily with gonadal responses, the potency of each extract may be judged from the per cent increase in ovarian weight which it produced in each instance. The results show that an increase in the oxygen consumption rate of immature rats given acid extracts of anterior pituitary gland was obtained in 9 out of 12 series of observations. It is also apparent that the extracts prepared from rat or sheep hypophyses contained more of the thyrokinetic factor than those from human autopsy material. The study of histologic sections

of the thyroid gland from many of the rats given varying doses of the pituitary extracts shows that in most instances no marked variation from the normal occurred in spite of the increased oxygen consumption (fig. 1). On the other hand, it has been possible to definitely produce hyperplastic changes, as shown by the absence of colloid and a change in the lining of the acini from a low cuboidal to a high columnar type of epithelium (fig. 2), in rats given a higher dosage of sheep anterior pituitary extract than those listed in this report. This histologic change was also observed in the thyroid glands of hypophysectomized rats which had been injected with the same preparation.

In table 2 are given the results obtained in 24 immature rats injected for periods varying from 5 to 20 days with gonad-stimulating extracts prepared from human pregnancy blood. It is seen that a very different effect was produced from that found with the pituitary extracts. In no

TABLE 2

Effect of gonad-stimulating extracts made from blood of pregnant women on the oxygen consumption of rats

EXTRACT	DURATION EXPT. DAYS	NO. OF RATS	AVE. BODY WEIGHT	PER CENT INCREASE WEIGHT OVARIES	AVE. O_2 CONSUMPT. RATE	PER CENT OF NORMAL RATE
Controls		12			0.61	100
xiv-3	5	9	41	175	0.43	70
xv-5	5	5	38	275	0.48	79
xviii-3	10	3	45	637	0.59	97
xv-15	15	5	49	613	0.59	97
xv-20	20	2	57	613	0.63	103

case has any significant increase in the rate of oxygen consumption occurred, and in fact there was a very definite diminution in the rate in experiments of 5 days' duration. The exact significance of this finding has not been determined, although it is possible that it is not due to an action on the thyroid but to a factor present in the extracts which affects metabolism directly. This extract has also been found ineffective in overcoming the atrophy of the thyroid gland resulting from hypophysectomy.

Thyroid feeding. The rats used in the thyroid feeding experiments were between 20 and 23 days of age at the beginning of each experiment, and were injected twice daily with 0.25 cc. of the extracts except on intervening Sundays when a single dose of 0.5 cc. was given. They were kept in groups of 5 to 9 in small cages, and known amounts of desiccated thyroid gland substance (Armour's) were thoroughly mixed with their feed. A rough estimate of the daily dose of thyroid substance taken by each rat could thus be made from the amount of feed consumed in each cage. As shown

in table 3, the thyroid substance was administered for from 1 to 5 days before the extracts were injected. In the majority of experiments, 250 mgm. of the thyroid substance were mixed with 100 grams of feed, and the approximate daily intake of each rat varied from 34 to 41 mgm. of thyroid substance per 100 grams of body weight. At this dosage, the oxygen con-

TABLE 3

Effect of gonad-stimulating extracts on ovarian and uterine weights of immature rats fed thyroid substance

EXTRACT	DAYS INJ'D	THYROID FEEDING		NO. OF RATS	AVE. BODY WEIGHT	AVE. GROSS WEIGHTS		PER CENT INCREASE IN WT.	
		Per cent in feed	Days fed			Uterus	Ovaries	Uterus	Ovaries
P.B.E. xx-6....	5	0.25	10	4	31	0.380	0.015	26	18
do	5	Controls		3	38	0.117	0.024	225	90
P.B.E. xviii-3..	5	0.25	6	4	27	0.076	0.019	136	64
do ..	5	Controls		6	25	0.098	0.023	225	118
P.B.E. xviii-3..	10	0.25	11	9	32	0.110	0.037	255	181
do ..	10	Controls		7	42	0.205	0.082	645	650
P.B.E. xx-3....	5	0.25	10	8	41	0.063	0.025	130	137
do	5	Controls		10	36	0.099	0.038	186	210
P.B.E. xxi-3....	5	0.5	8	8	26	0.054	0.019	77	73
do	5	Controls		5	28	0.112	0.028	261	145
P.B.E. xiv-3....	5	1.0	6	10	32	0.055	0.013	78	0
do	5	Controls		9	41	0.092	0.029	240	162
S 15-2.....	4	1.0	6	9	31	0.058	0.022	85	82
do	4	Controls		7	32	0.053	0.039	70	209
S 24-3.....	5	0.25	10	10	34	0.035	0.033	3	180
do	5	Controls		7	38	0.061	0.068	68	430
S 32.....	5	Thyroxin		6	29	0.046	0.025	70	127
do	5	Controls		8	26	0.059	0.053	90	382

"P.B.E." refers to extracts prepared from blood of pregnant women, and "S" to extracts made from anterior pituitary glands of sheep.

sumption rate was found increased as much as 80 per cent in 5 days in a number of cases. The animals generally supported this dose readily and continued to increase in weight, although in 10-day experiments a small number became ill and died. When 0.5 or 1.0 per cent of thyroid substance was introduced in the feed the daily dose amounted to from 60 to 110 mgm.

per 100 grams of body weight and there was not only a serious interference with body growth but even a loss of weight, the rats developed diarrhea, and there was a comparatively high mortality rate.

The series of rats given thyroxin received three injections of this preparation, the total dose amounting to 1.9 mgm. per 100 grams of body weight. These injections were given on the first, third, and fifth days of the experiment, while the anterior lobe extract was administered twice daily for five days, beginning on the second day. The rats tolerated the thyroxin very well, continued to increase in weight and were in good condition at the end of the experiment.

The controls for each series were littermates of the experimental animals, and were kept under identical conditions in adjacent cages. They received the same feed except, of course, for the absence of thyroid substance.

A total of 130 immature rats was used, and 9 series of experiments were conducted. Forty-three thyroid-fed immature rats and 40 littermate controls were given various pregnancy blood extracts, while 47 rats, of which 19 were fed thyroid substance, 6 were given thyroxin, and 22 were littermate controls, received sheep anterior lobe preparations.

The results are given in table 3, and a comparison of the per cent increase in ovarian and uterine weights induced in the experimental as compared with the control rats, shows a remarkable constancy. In every case the ovaries, and with only one exception the uteri, of the animals receiving thyroid substance or thyroxin failed to increase in weight to the same extent as the controls.

It already has been pointed out that definite characteristics may be demonstrated in the histologic appearance of the ovaries of immature rats given the sheep anterior pituitary extract used in these studies (Fluhmann, 1933a). In these experiments, it could not be shown that the administration of thyroid substance altered the appearance of the ovaries of the animals given this preparation, in spite of the lessened increase in weight. The ovaries of both thyroid-fed, thyroxin, and control rats presented the usual picture of numerous small corpora lutea and medium-sized follicles in various stages of chromatolytic degeneration.

The ovaries of the thyroid-fed rats given the pregnancy blood extracts also usually presented the same histologic appearance as those of the controls, namely, large corpora lutea, lutein cysts and occasional developing graafian follicles (fig. 3). In some instances, however, a very remarkable change was observed, in that there occurred an extensive luteinization of the theca interna of small follicles resulting in an excessive production of interstitial tissue (fig. 4). This transformation was not usually accompanied by any great increase in ovarian weight, but in one experiment the ovaries of 4 rats gave increases of over 275 per cent in weight, and numerous sections taken at different levels failed to show a single corpus luteum,

lutein cyst or normal graafian follicle while there was the above-described tremendous production of interstitial tissue. The factors controlling this phenomenon are not clear, but it would seem that it may be brought about

TABLE 4

Effect of gonad-stimulating extracts on ovarian and uterine weights of immature rats subjected to various operations on the thyroid gland

OPERATION	DURATION EXPT. DAYS	EXTRACT	NO. OF RATS	AVE. BODY WEIGHT	AVE. GROSS WEIGHTS		PER CENT INCREASE IN WEIGHT	
					Uterus	Ovaries	Uterus	Ovaries
T-P	5	S 15	8	26	0.066	0.041	109	273
Controls	5	S 15	7	32	0.053	0.039	70	209
T-P	10	S 19	3	31	0.122	0.079	303	536
Controls	10	S 19	10	62	0.139	0.069	225	312
Ti-P	10	S 18	7	31	0.121	0.044	290	290
Controls	10	S 18	4	45	0.068	0.019	135	62
Ti	5	S 6 M	3	37	0.072	0.030	103	130
Ti	10	S 6 M	4	36	0.142	0.049	310	210
Ti	15	S 6 M	4	64	0.213	0.074	383	412
Controls	16	S 6 M	3	67	0.150	0.039	233	162
Ti	12	S 26	6	39	0.143	0.099	293	610
Controls	12	S 26	4	41	0.107	0.054	295	400
Ti	15	S 30	10	41	0.082	0.027	200	150
Controls	15	S 30	7	38	0.046	0.018	29	40
Ti	9	S 34	7	38	0.116	0.117	225	810
Controls	9	S 34	6	42	0.130	0.087	370	687
T-P	5	P.B.E. xiv	7	32	0.122	0.028	288	118
Controls	5	P.B.E. xiv	9	41	0.092	0.029	240	162
Ti	9	P.B.E. xxii	9	48	0.169	0.062	465	412
Controls	9	P.B.E. xxii	7	50	0.168	0.065	445	425

T-P, thyro-parathyroidectomy; Ti-P, partial thyroidectomy and parathyroidectomy; and Ti, partial thyroidectomy.

"S" refers to extracts prepared from sheep anterior pituitary glands, and "P.B.E." to extracts made from blood of pregnant women.

by either diminishing the dosage of the pregnancy blood extract or increasing that of the thyroid substance. This finding is of particular interest in view of the production of a similar histologic change with similar extracts

in the ovaries of hypophysectomized (Noguchi, 1933; Collip, Selye and Thomson, 1933; Leonard and Smith, 1933), or very young rats (Selye and Collip, 1933).

Thyroidectomy. In this group of experiments rats of the same age as those for the thyroid feeding were employed, and the injection of the extracts was carried out in the same manner. The operations on the thyroid were performed under ether anesthesia. The thyroid was exposed by a midline incision in the neck and was removed by dissection with fine scissors, while hemorrhage was controlled solely by pressure with small absorbent cotton pads. Considerable difficulty was experienced in determining positively whether the gland had been completely removed or not, but a low oxygen consumption rate was obtained in the experiments with extracts S-15 and S-19 and P.B.E. xiv, demonstrating at least that a definite hypo-thyroid condition resulted. In the experiment with S-18, the oxygen consumption rate of 4 experimental animals was markedly increased and in 2 cases small fragments of remaining thyroid tissue were demonstrated in serial sections. In the other experiments no attempt was made to do a complete excision, and a portion of one lobe of the thyroid as well as one parathyroid gland were not removed. A high post-operative mortality rate resulted from attempts at complete thyroidectomy in these young rats. The injection of the extracts was begun on the day after the operation had been performed.

The control animals were littermates of the experimental rats and were kept under identical conditions in the same cages. They were, in addition, subjected to an ether anesthesia while an operation on the neck was performed and the thyroid gland simply exposed. One or two superficial vessels in the neck were also incised in order to induce a hemorrhage such as occurred when a thyroidectomy was done.

A total of 68 experimental and 57 control rats was employed. The results are given in table 4, and it is seen that in all instances the animals subjected to partial or complete thyroidectomy gave a greater per cent increase in ovarian weight than the controls receiving the same dose of the sheep anterior lobe extract. On the other hand, no significant difference was observed when the pregnancy blood extract was administered.

The study of numerous microscopic sections from the ovaries of these rats shows that any difference between experimental and control rats is solely in the size and weight of the ovaries, and no significant histologic variations could be demonstrated.

Discussion. It thus has been shown that the administration of thyroid substance or thyroxin to immature rats partly inhibits the increase of ovarian weight which is induced by gonad-stimulating hormones. Conversely, the effect on ovarian weight of an extract which is both gonad-stimulating and thyroid-stimulating, such as the sheep anterior lobe

preparation used in these studies, is definitely increased after partial or complete thyroidectomy. These findings may be interpreted as supporting the conception that the over-active thyroid gland inhibits some phases of ovarian function, either directly, or indirectly as a result of variations in basal metabolism.

The mechanism by which this inhibition is brought about is not clear, except that it is due to a direct effect on the ovary and not indirectly through the anterior hypophysis, since it was manifested when anterior lobe extracts were employed. It likewise does not seem likely that the suggestion of Weichert (1930) and Van Horn (1933) in the case of interference with estrin action, that the increased basal metabolism leads to such rapid excretion of the hormone that only small amounts are available, is a possibility in this case. If rapid excretion of the hormone is the sole explanation, it would be assumed that the extensive thecal luteinization noted in some instances where the pregnancy blood extract was employed, was due to the action of small amounts of the extract. In a previous report (Fluhmann, 1933a), the effect of subminimal doses of pregnancy blood extract on the ovaries of immature rats was reported and in no case did the histologic changes resemble those found in this instance.

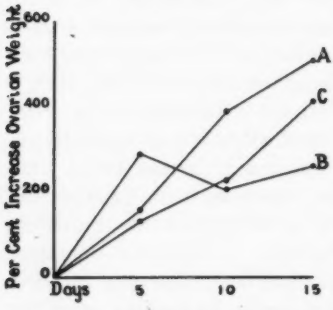

Fig. 5. Effect on the weight of the ovaries of small groups of immature rats given daily injections of gonad-stimulating extracts for periods of 5, 10, and 15 days. *A* shows the increase obtained by the use of a pregnancy blood extract (no. xiii) in normal animals. *B* is for a group of normal rats given daily injections of a sheep anterior pituitary extract (no. S.A.P.E. 201). *C* gives the changes resulting from the administration of a similar anterior lobe preparation (no. S6M) to rats in which a partial thyroidectomy had been first performed.

These experiments were originally undertaken in the hope that they might cast some light on a fundamental difference in the effect on ovarian weight induced by various gonad-stimulating hormones (Fluhmann, 1932b, 1933b, c). It was found that whereas pregnancy blood extracts had a cumulative action and the ovaries of immature rats progressively increased in size as the period of administration was prolonged, acid sheep anterior pituitary extracts, at doses which stimulate increases of from 100 to 500 per cent in ovarian weight in 5 days, failed to produce any further increase even when the daily injections were continued for 20 days. The work of Loeb and Friedman (1931) and of Loeb (1932) in guinea pigs suggested the existence of a relationship between the responses of the thyroid and the gonads, and it seemed of importance to determine if an influence from the thyroid

was responsible for the differences in ovarian weight noted in the above experiments.

Since it has been shown that the sheep anterior pituitary extract has a definite stimulating effect on the thyroid gland and the pregnancy blood extract has a depressing influence, it seems logical to conclude from the results of these studies that the differences in ovarian response after prolonged periods of injection *are* due to the thyroid. The effect on ovarian weight of a sheep anterior lobe extract in normal and in partially thyroidectomized rats and of a pregnancy blood extract in normal animals, is shown in figure 5 and demonstrates the similarity of the curves produced by the two hormones when the anterior lobe extract is given to thyroidectomized rats. The observation that with large doses of sheep pituitary extract the inhibition of the increase in ovarian weight only persisted for 10 days and was then succeeded by a period of excessive growth (Fluhmann, 1933c), also finds a ready explanation in terms of thyroid function. During the first ten days a condition of hyperthyroidism existed, as suggested by the loss of body weight, diarrhea, and general ill health, and this was then succeeded by a period of hypothyroidism or of normalcy when there was a marked increase in body weight and a tremendous increase in the size of the ovaries. A similar return to normal of the thyroid gland after a period of excessive stimulation by anterior lobe hormone has been previously mentioned by Collip (1933).

The observation that the effect of one anterior lobe hormone may be indirectly influenced by another anterior lobe factor which stimulates a third gland is of vital importance in fundamental studies of the hypophysis. Since the "master gland" apparently produces many hormones it will be essential not only to wait for purified extracts of each element before their true function can be accurately determined, but also to hesitate about accepting any alleged new hormone until it is proved that its effect is not an indirect result of the stimulation or inhibition of some other endocrinological factor in the test animal.

SUMMARY

1. The administration of acid gonad-stimulating extracts made from sheep, rat or human anterior hypophyses resulted in an increase of the rate of oxygen consumption of immature white rats. With larger doses the sheep anterior lobe extract produced a hyperplasia of the thyroid gland.

2. The injection of an acid gonad-stimulating extract made from blood of pregnant women resulted in a diminution of the rate of oxygen consumption in 5-day experiments, but no marked deviation from the normal occurred when this preparation was given for from 10 to 20 days.

3. The feeding of various amounts of desiccated thyroid substance or

the administration of thyroxin to immature rats injected with acid gonad-stimulating extracts made from either sheep anterior hypophyses or human pregnancy blood, resulted in a markedly lessened increase of uterine and ovarian weight.

4. There were no demonstrable histological differences between the ovaries of the control and thyroid-fed animals given the sheep anterior lobe extract.

5. In some instances, the injection of the extract made from blood of pregnant women to immature thyroid-fed rats resulted in an extensive production of interstitial tissue in the ovaries.

6. The administration of a gonad- and thyroid-stimulating extract made from anterior pituitary glands of sheep to immature rats produced greater increases of ovarian weight when a partial or complete thyroidectomy had been first performed. This is considered as further evidence that the over-active thyroid inhibits ovarian function.

7. The injection of a gonad-stimulating extract prepared from blood of pregnant women to immature rats did not produce a greater increase of ovarian weight when a partial or complete thyroidectomy had been first performed.

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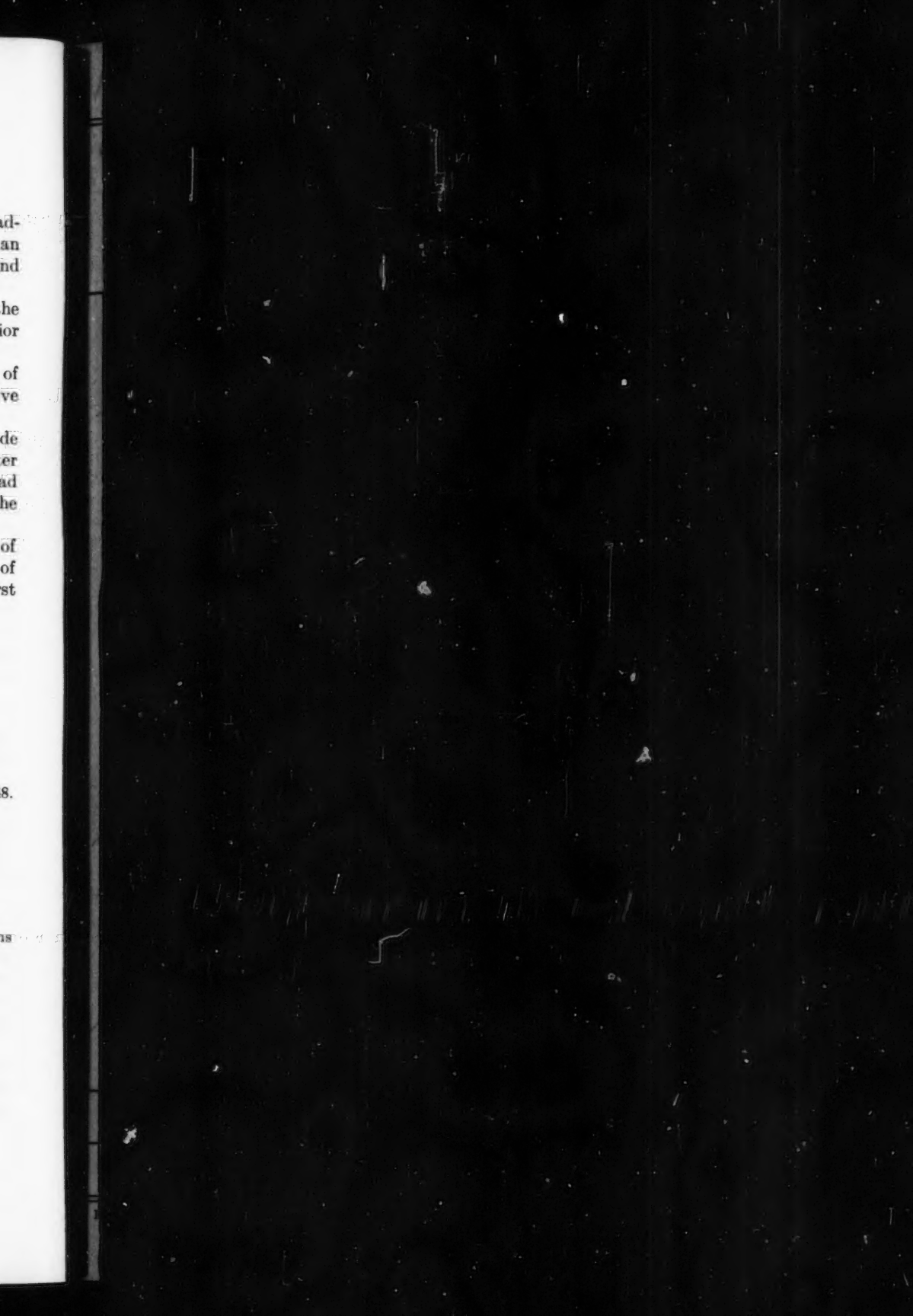
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